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AN ECTOPARASITE (ACARINA: MESOSTIGMATA) FROM MOTHS OF THE GENUS *ZALE*

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Unilateral destruction of the auditory organ in several species of noctuid moths by the widely distributed mite *Myrmonyssus phalaenodectes* has been reported previously (Treat, 1954, 1955). The mite to be described here, though also associated with noctuid tympana, feeds from other parts of the host, leaving the auditory apparatus undamaged. The infestations thus far discovered are bilateral; eggs and early stages are found in the external tympanic recesses of both sides. The tympanic membranes are not perforated, and the countertympanic cavities (small in this genus of moths) are unoccupied. Nymphs and adult mites of both sexes have been taken from among the scales forming the deep vestiture of the thoracic dorsum, from beneath the tegulae, where feeding scars have been noted, and from the metascutellar region, where cast skins have also been found.

The mites are described from dead and dried specimens discovered on pinned moths in the LEPIDOPTERA collection of the American Museum of Natural History. Among more than 500 representatives of *Zale* Hübner and related genera which were examined, only 11 were found to be infested. It is possible that some infestations were overlooked, since the mites are not always in positions where they can be readily seen without some damage to the host. The dry mites and their eggs were mounted directly in Hoyer's medium (Baker and Wharton, 1952), and the slides were gently heated to relax and clear the specimens. Remounting was usually necessary because of air bubbles released from the specimens by the heat.

Though the living mites have not been seen, their character as true parasites rather than scavengers or phoretics may be confidently inferred from the finding of their feeding scars and of all developmental stages on the same host. The weak sclerotization and the virtual disappearance of the tritosternum are structural features which, in this group of mites, are associated with the parasitic habit.

Host determinations and collection data are given below as they appear in the collection of the American Museum of Natural History.

Zale lunata Drury, female, "Speonk, L. I., N. Y., July 16, 1950, Leory Wilcox"
Zale lunata Drury, female, "Thomasville, Ga., Thomas County, ix–30–1950, A. H. & S. K. Rindge; Collection of Fred H. Rindge"
Zale lunata Drury, female, "Laurel, Miss., Jones County, ix–28–1952, A. H. & S. K. Rindge; Collection of Fred H. Rindge"
Zale lunata Drury, female, "Mobile, Alabama, Mobile County, x–2–1951, A. H. & S. K. Rindge; Collection of Fred H. Rindge"

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Zale lunata saundersii Bethune, female, "Riverhead, L. I., N. Y., 18 June, 1954; Roy Latham"

Zale lunata edusa Drury, male, "Glassboro, N. J., VI 30; J. B. Smith Collection, Rutgers"

Zale fictilis Guenée, female, "Port Sewell, Martin County, Fla., 28-1-39; Collectors L. C. Sanford, L. J. Sanford"

Zale fictilis Guenée, male, "Port Sewell, Martin County, Fla., I, 19-23, 1950"

Zale fictilis Guenée, female, "Port Sewell, Fla., 2-4 II 39; Collectors L. C. Sanford, L. J. Sanford"

Zale undularis Drury, female, "Riverhead, L. I., 30 May, 1954, Roy Latham"

Zale obliqua Guenée, male, "Manahawkin, Ocean County, New Jersey, July 6-7, 1948 (at neon light), L. J. Sanford"

This acarine will be readily recognized as belonging to the gamasine Superfamily PARASITOIDEA Banks, as revived by Camin and Gorirossi (1955). The vestigial tritosternum, however, in association with the long peritremes and the absence of a fixed cheliceral digit, distinguishes these mites from those of any previously described family. Future work will no doubt clarify the relationships of the gamasine mites and reduce the status of many of the groups now regarded as of family rank. For the present, however, there appears to be no alternative to proposing new groups as follows:

Family OTOPHEIDOMENIDAE, NEW FAMILY

Diagnosis: palpal tarsus with two-tined special seta; tritosternum vestigial or absent in both sexes and all stages; 3 pairs of sternal setae; peritremes of female extending beyond anterior borders of coxae II. Ectoparasitic upon insects.

Type genus: *Otopheidomenis gen. nov.*

Otopheidomenis

Diagnosis: Otopheidomenidae: Fixed digit of female chelicera absent or greatly reduced; movable digit denticulate; dorsal plate deeply notched near middle of lateral margins. Eggs laid in or near external tympanic recess of noctuid moths.

Type species: *Otopheidomenis zalelestes*, sp. nov.

Otopheidomenis zalelestes gen. nov., sp. nov.

DESCRIPTION

Female (Plate I, a). Palpi with 5 segments, the palpal tarsus bearing a prominent 2-tined seta. Tectum smooth, bluntly rounded. Movable digit of chelicera about 45 microns long, slightly upcurved, tapering to a sharp point, and bearing minute denticles (Plate I, b). Fixed digit absent or greatly reduced. Hypostome with slender, incurved lanceolate corniculi. Salivary styli well developed. Deutosternum with 12 rows of 8 to 10 minute teeth, detectable only with phase contrast microscope. Tritosternum absent or represented by minute elliptical plate only. Idiosoma oval; length averaging 556 microns; greatest width, between peritremes, about equal to greatest depth in mounted specimens, averaging 308 microns. All sclerotization very weak. Sternal plate bordered by 2 pairs of setae, a 3rd pair arising from slight sclerotizations between coxae II and III. Genito-ventral plate broadly rounded at rear and flanked by a pair of setae. Spatulate anterior portion of genito-ventral plate extending forward to overlap rear margin of sternal plate, slightly broadened between coxae IV, and reinforced medially with a broad, blunt keel margined with dentate sclerotizations. Anal plate with a median dorsal seta and a pair of ventro-lateral setae. Dorsal plate (Plate I, c) notched at peritremal level and covering most of idiosoma; a pair of prominent, rearwardly directed setae at its posterior margin. Peritremes kinky in outline, extending well beyond anterior margin of coxae II, length averaging 215 microns. A row of 4 setae on striated surface of venter about midway between genito-ventral and anal plates. All legs with pretarsi, caruncles, and minute claws. Average leg measurements, in microns: I, 488; II, 476; III, 486; IV, 537. A single seta on coxa IV. Tarsus I with a dorsal patch of special, short, blunt, non-striated setae. Dried specimens yellowish white, flattened, and slightly arched at notch of dorsal plate. Specimens mounted and cleared in Hoyer's medium faintly yellow and highly transparent. Living mites not seen. Described from 30 specimens.

Male (Plate I, d). Gnathosome usually directed ventrally; only rarely seen extended as in

figure. Palpal tarsus with 2-tined seta. Chelicera with special copulatory apparatus (Plate I, e) consisting of a hooked process, a grooved member terminating in an oval pad, and a blunt stylet. Corniculi and salivary stylets present. Length of idiosoma averaging 465 microns; width 293, depth, 231 microns. Genital orifice at or near anterior border of sternal plate, which bears 3 pairs of setae. Ventral and anal plates as figured. Dorsal plate as in Plate I, f. Average leg measurements in microns: I, 315; II, 300; III, 296; IV, 312. Peritremes between coxae III and IV, length averaging 62 microns. 11 specimens.

Egg (Plate II, a). Laid singly or in loose clumps adhering lightly to scales or setae within the tympanic hood of the host, or more rarely on the thoracic dorsum. When dry, shriveled, yellow or whitish; when mounted and cleared, slightly elongate and often somewhat bean-shaped as in figure. With clear cortical layer and inclusions of various sizes. Average length 279, width 195 microns. 70 specimens.

Embryo (Plate II, b). As figured. 4 specimens.

Larva (Plate II, c). Peritremes absent; cuticle extremely thin and transparent. Movable digits of chelicerae (Plate II, d) with fewer denticles than adult, usually 2 or 3. Average length of idiosoma, 286, width 203 microns. Legs: I, 251; II, 234; III, 226 microns. 20 specimens.

Nymphs (Plate II, e and f). Idiosoma varying in length from 262 to 493 microns, in width from 200 to 263. Since the only molting specimens observed were emergent females, and since the size variation in the nymphs is practically continuous, it is possible that there is but a single nymphal instar. The ratio of leg length to length of idiosoma is nearly constant throughout the series of nymphal specimens. Peritremes range in length from 15 to 53 microns. Feeding organs resemble those of the adult. 23 specimens.

The holotype is a gravid female from a specimen of *Zale lunata saundersii* Bethune, female, taken on 18 June, 1954, at Riverhead, Long Island, New York, by Roy Latham. The allotype male is from *Zale lunata lunata* Drury, female, taken on 16 June, 1952, by Leroy Wilcox at Speonk, Long Island, New York. The types together with 2 paratype females, 2 males, and representative early stages, are deposited in the American Museum of Natural History. Additional paratypes are in the collections of the United States National Museum, of Dr. G. W. Wharton of the University of Maryland, and of the author.

The generic name *Otopheidomenis* is derived from the Greek οὖς—ear, plus φείδομαι—to spare, or to draw back or turn away from. The trivial name *zalelestes* is taken from *Zale*, the generic name of the hosts in which the mites were discovered, plus the Greek λῃστής—a robber, plunderer, or pirate.

DISCUSSION

The mite here described has structural features resembling a number of gamasine families. Its general conspectus suggests either the hypoaspidine laelaptids or the phytoseids, from both of which, however, it is clearly separated by the great reduction of the tritosternum. The comparatively few families which lack the tritosternum are all parasites of vertebrates. They differ from *Otopheidomenis* in the form of the feeding organs and also in having reduced peritremes.

In its use of the external tympanic recess of a noctuid moth as a place for oviposition, this mite affords an interesting comparison with the moth ear mite *Myrmonyssus phalaenodectes*. The latter is evidently a parasite well adapted to the exploitation of the tympanic organs as a site for both feeding and breeding. Although *M. phalaenodectes* invades and occupies the tympanic air sac and countertympanic cavity, destroying the auditory apparatus as it does so, it quite consistently limits its depredations to one side of the moth, leaving the insect but little impaired in its ability to detect and respond to sounds. Host species of *M. phalaenodectes* range through four of the noctuid subfamilies, although the incidence of the parasite appears to be high in only one of these, the HADENINAE, and chiefly in certain genera of that group.

Otopheidomenis, by contrast, does not enter the internal cavities of the tympanic

apparatus. Its feeding sites are more conventional ones—the articular membranes at or near the wing bases. Sparing, as it does, the auditory sensitivity of its host, it can afford to occupy both sides of the moth rather than one side only. This coincidence calls attention to the unsolved problem of identifying the circumstances which so effectively restrict the *M. phalaenodectes* colony to unilaterality.

The eggs of *M. phalaenodectes* are usually cemented to the host's cuticle, which is punctured by the female preparatory to the placing of the egg, and which shows a more or less distinct scar when the egg is removed. The eggs of *Otopheidomenis*, on the contrary, are but lightly attached, often to the hairs or scales within the tympanic hood. They leave no mark or scar when they are displaced. The mites of both species tend to go upward and forward when they eventually leave the tympanic region. *In Otopheidomenis*, this migration evidently begins immediately after the hatching of the eggs, while in *M. phalaenodectes* it is delayed until development has been completed or nearly completed within the broached tympanic air sac and countertympanic cavity. In *M. phalaenodectes*, the feeding of young females in the patagial region appears to be incidental to their preparation for leaving the host. They are not usually found under the tegulae, where nymphs and females of *O. zalestes* often congregate. The anatomy of the feeding organs is significantly different in the two species, those of *M. phalaenodectes* perhaps adapted to cutting and chewing rather than exclusively to piercing and sucking as would seem to be the case with *O. zalelestes*.

It is of interest that *Otopheidomenis* has thus far been found only in a limited group of moths of a subfamily (CATOCALINAE) which has as yet yielded no infestations of *M. phalaenodectes*. The host collection data given above indicate that the mite, though perhaps rare, is by no means of strictly local occurrence. Forbes (1954) states that the host genus *Zale* is "confined to America." The mite, of course, may eventually be discovered on insects of other genera.

Although the two species of mites are perhaps not closely related, it is entertaining to speculate upon the possibility that their different ways of life represent successive stages in the evolution of the parasitic relationship. The weak sclerotization of *Otopheidomenis* suggests that it is not a newcomer to the habit of parasitism, though it has apparently never taken the short step from strictly ectoparasitic existence to the invasion of the tracheal system via the tympanic membrane. Certain similarities between this mite and the lung mites of various vertebrates suggest the further possibility that some internal parasites may have been acquired by their vertebrate hosts from among the ectoparasites of the animals upon which they prey.

SUMMARY

A new family, genus, and species of gamasine mite is described from specimens found as ectoparasites upon moths of the genus *Zale* collected in New York, New Jersey, Georgia, Florida, Alabama, and Mississippi.

ACKNOWLEDGMENTS

The warmest thanks of the author are extended to Dr. George W. Wharton of the University of Maryland for much help and advice in the study and description of the collected mites and for reviewing the manuscript, to Dr. Fred H. Rindge of the American Museum of Natural History for making available the Lepidoptera

collection of that institution for study, and to Dr. Israel E. Drabkin of The City College of New York for his learned and expert assistance in the construction of the scientific names.

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Description of Plates

Plate I

Fig. a. *Otopheidomenis zalelestes* n. sp., ventral view of female with chelicerae exserted. Drawn freehand from several specimens. Cuticular striations are suggested but not shown in full detail.

Fig. b. terminal portion of left chelicera of female, ventral view.

Fig. c. dorsal plate and tectum of female.

Fig. d. ventral view of male with chelicerae exserted and elevated. Drawn freehand from allotype male.

Fig. e. terminal portion of left chelicera of male.

Fig. f. dorsal plate of male.

Plate II

Fig. a. *Otopheidomenis zalelestes*, egg.

Fig. b. embryo.

Fig. c. ventral view of larva.

Fig. d. feeding organs of larva, seen from left side.

Figs. e, f. ventral views of nymphs.

PLATE I

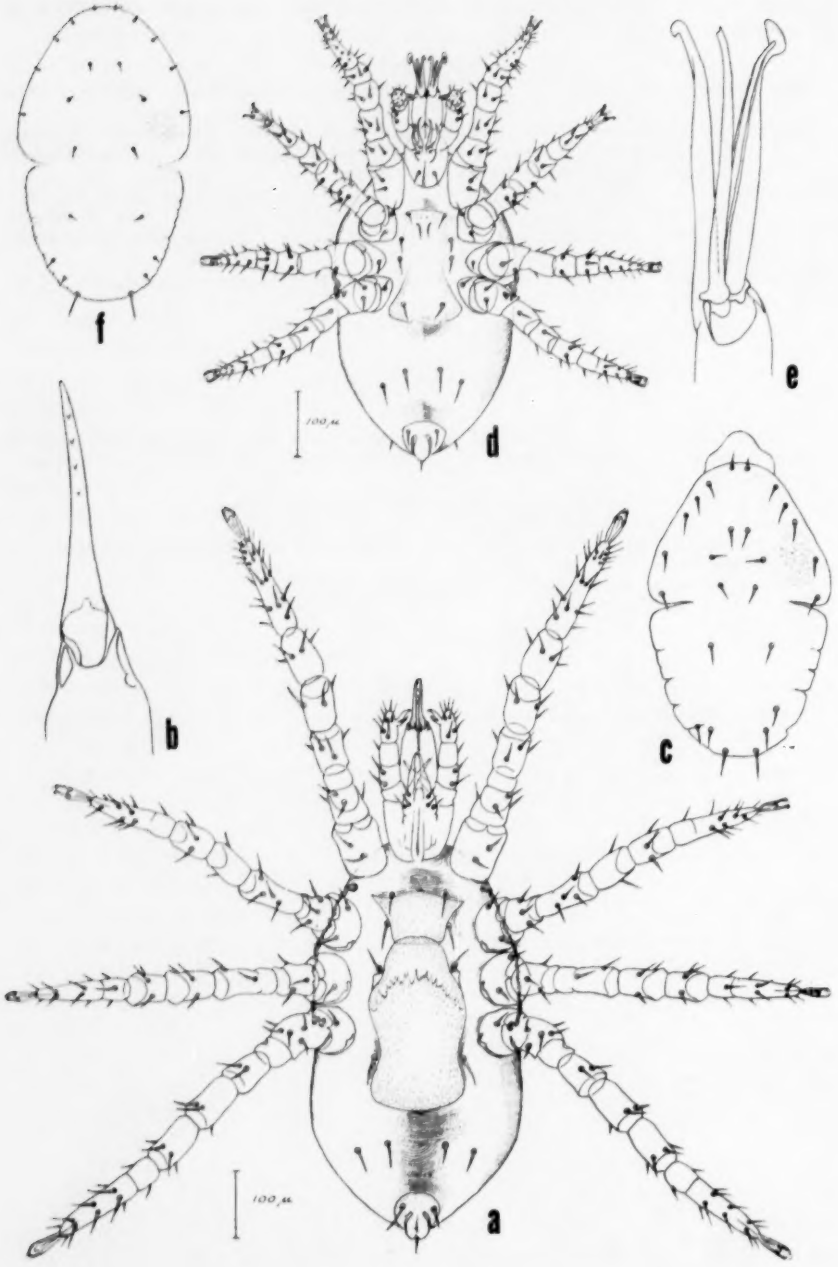
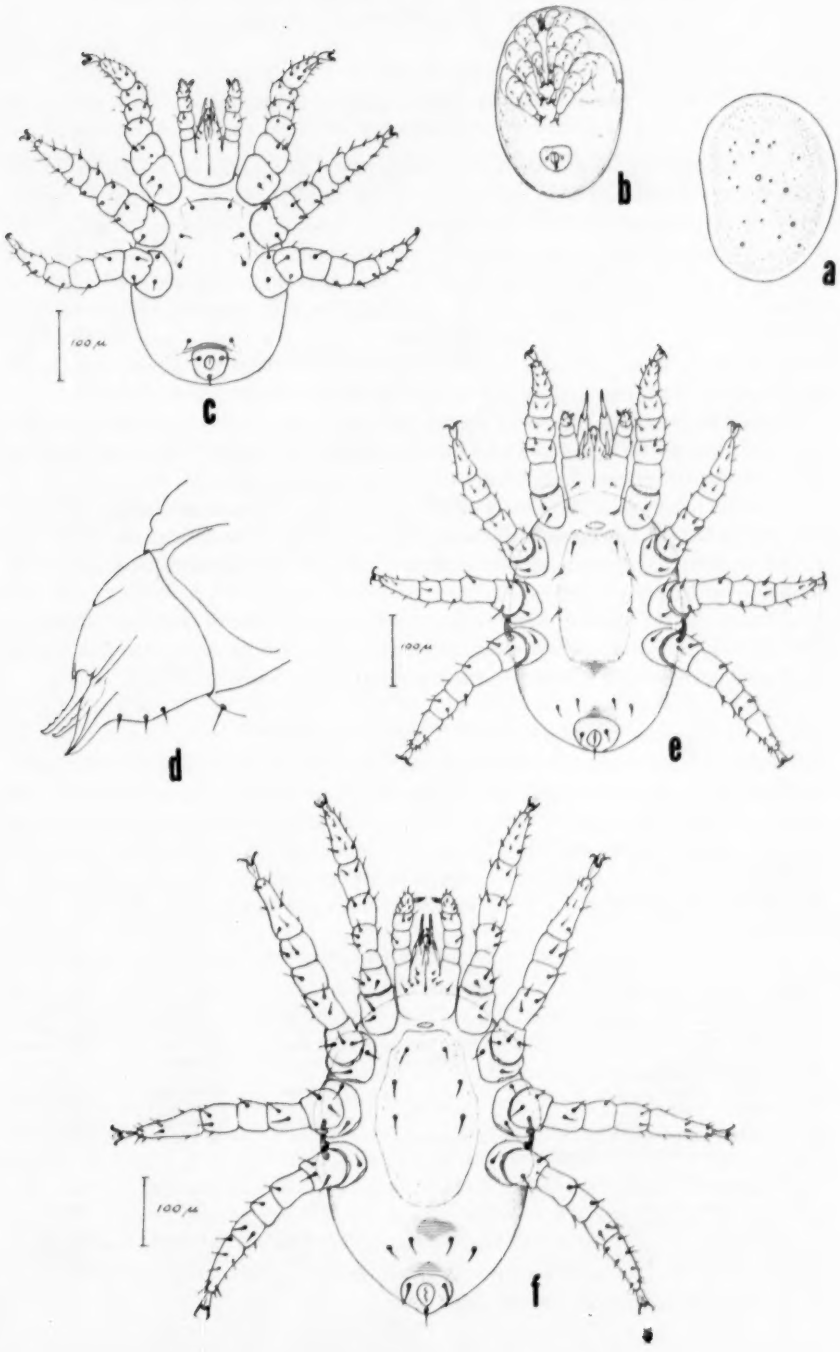


PLATE II



TRYPANOSOMIASIS IN THE COLLARED LEMMING, *DICROSTONYX TORQUATUS* (RODENTIA)

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The collared lemming, *Dicrostonyx torquatus*, has a holarctic range which extends farther northward than that of any other small mammal. During a current study of the cytological basis of adaptation to cold in collared lemmings, I discovered trypanosomes in the blood of four specimens. Trypanosomiasis has not been previously reported in lemmings and the natural occurrences and effects of trypanosomes in wild populations of mammals are not frequently observed (see Doran, 1954). This report presents data on the abundance, morphology, and biologic effects of the lemming trypanosome, and also describes attempts to locate the reproductive forms and an attempt to inoculate another host.

All of the lemmings used in this study were obtained by the writer within one-half mile of the town of Churchill, Manitoba, in August, 1953 and in July, 1954, or are laboratory descendants of animals so obtained.

I am grateful to the Horace H. Rackham School of Graduate Studies, University of Michigan, for funds supporting the obtaining of lemmings in 1954, and to the Upjohn Company and Dr. Burton L. Baker for the rats used in experimental inoculations.

The use of the name *torquatus* for the species of collared lemming occurring at Churchill, Manitoba, follows the suggestion of recent authors that this animal is conspecific with the palearctic species *torquatus*, (Rausch, 1953).

INCIDENCE AND COURSE OF INFECTION

Blood smears which were briefly air-dried, fixed in methanol and stained with Wright's stain were taken from 34 collared lemmings. In smears from four adult lemmings killed a few hours after capture at Churchill, one lemming was shown to have trypanosomes. Three of 9 lemmings killed by ether anesthesia after experiments in the laboratory showed trypanosomes. Thirteen adult and 8 young lemmings which died in captivity from various causes did not show

TABLE I.—*Data on the infected lemmings*

Lemming number and sex	4-B-78♂	34♀	11♀	61♂
Age at time of death	adult	adult	adult	6 mos.
Body-weight and total length at time of death	? 130 mm.	48.0 gms 135 mm.	56.7 gms 150 mm.	41.4 gms. 129 mm.
Length of captivity	0 days	5 mos.	6 mos.	6 mos.
Time since last contact with another animal	?	49 days	106 days	0 days
Environment condition during month before death	natural summer	24 hrs. light/day, 18° F.	0 hrs. light/day, 64–77° F.	24 hrs. light/day, 75° F.
Blood vol. (per cent) = erythrocytes, (1 hr. at 3800 r.p.m.)	?	26	38	?
Erythrocytes/cmm. blood	?	6.45×10^6	11.53×10^6	9.88×10^6
Leucocytes/cmm. blood	?	889	2288	6688
Trypanosomes/erythrocytes in blood smears	17/1008	25/2787	0+/4167	13/4361
Trypanosomes/cmm. blood	?	58,000	< 2,000	29,000

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trypanosomes. The significance of the incidence of trypanosomes in the captive lemmings is uncertain since infection during captivity might be possible, although the animals were periodically dusted with a powder to kill ectoparasites. Data on the four infected lemmings are presented in Table I. It is apparent in the case of specimen #11 that the trypanosomes can be present in the blood more than three months after initial infection, since this animal was not in proximity to another animal or possibly infectious carriers during this period. Lemming #11 gave birth to #61 enroute from Churchill and it is interesting that #61 was found to be infected 6 months later. It seems most probable that infection is transmitted by ectoparasites, although evidence is lacking.

Blood smears from other small mammals (1 *Synaptomys borealis*, 3 *Microtus pennsylvanicus*, and 1 *Zapus hudsonius*) caught at Churchill failed to show trypanosomes.

DESCRIPTION OF THE TRYPANOSOME

Examination of diluted and oxalated fresh blood from specimen #34 demonstrated adult trypanosomes in active movement. Measurements of 100 trypanosomes from each of two infected lemmings (34 and 4-B-78) were made using blood smears which had been briefly air-dried, fixed in methanol, and stained with Wright's stain. The measurements were determined with dividers on camera lucida drawings of 4600× magnification (97× objective, 25× ocular). The points of the dividers were 2.3 mm. apart, corresponding to 0.5 micron in the original specimen. The means, standard deviations, and ranges of the measurements (in microns) are as follows (data from specimen 4-B-78 precede those from 34 in each instance): posterior end to center of kinetoplast: 3.78 ± 0.54, 1.7 – 4.8; 4.39 ± 0.50, 3.4 – 5.5; center of kinetoplast to center of nucleus: 7.11 ± 0.66, 3.2 – 8.5; 7.90 ± 0.47, 6.8 – 8.9; center of nucleus to anterior end: 11.22 ± 1.56, 7.0 – 14.3; 12.18 ± 1.21, 7.5 – 14.9; anterior end of body to end of free flagellum: 8.26 ± 1.72, 4.1 – 12.7; 6.95 ± 1.06, 3.1 – 10.6; total length, the sums of the preceding measurements are 30.37 ± 2.57, 25.8 – 33.3; 31.42 ± 1.18, 22.4 – 33.5. The trypanosome is tapered at both ends, and is usually so slender anteriorly that it is difficult to decide where the free flagellum begins. The nucleus, centrally located, usually about 2 microns long, often contains spheroidal chromatin granules of nearly uniform size surrounding a central pale area. The kinetoplast, averaging 0.7 microns wide and 0.5 long, varies from spheroidal to elongate and frequently has a constriction in the middle, in the axis of the trypanosome. The kinetoplast usually lies at right angles to the axis of the trypanosome, but is sometimes slanted nearly 45 degrees from the transverse position. It characteristically extends from membrane to membrane across the posterior tip of the trypanosome. The exact relation of the flagellum to the kinetoplast is difficult to determine; a gap or an attenuated segment of the flagellum frequently appears immediately anterior to the kinetoplast. The cytoplasm of most of the trypanosomes appears pale and structureless, and in others it stains blue and contains pale, ovoid vacuoles, particularly in the area between the nucleus and the kinetoplast. An undulating membrane and free flagellum are well-developed. The adult trypanosome of the lamming, *Dicrostonyx*, is similar in size and morphology to *Trypanosoma microti* (Laveran and Pettit, 1909; Lavier, 1921; Coatney, 1935) and *T. zapi* (Davis, 1952).

LIFE CYCLE IN THE LEMMING

No immature forms nor dividing trypanosomes were found in the blood smears from the infected lemmings. After death from ether anesthesia, the infected lemmings were fixed in either 10% formalin or in Bouin's fluid. From two or three of the infected animals, usually specimens 34 and 61, blocks of tissue were taken from muscle of the diaphragm, masseter muscle, tongue, ventricle of the heart, lung, liver, spleen, pancreas, submaxillary gland, stomach, caecum, kidney, urinary bladder, adrenal gland, and cerebral cortex. Serial paraffin sections 7 or 10 microns thick were prepared from these blocks of tissue and were stained by the following techniques: (1) Ehrlich's acid hematoxylin and eosin, (2) Mallory's phosphotungstic acid hematoxylin, (3) Heidenhain's iron hematoxylin, (4) Azure-eosinate, and (5) Wolbach's Giemsa (Lillie, 1948). Careful microscopic examination of the stained tissue sections failed to reveal immature or reproductive types of trypanosomes.

EXPERIMENTAL INOCULATION OF RATS

Five adult male Sprague-Dawley rats (298–480 gms.) were injected subcutaneously with diluted fresh blood from lemming 34. Blood smears from the tip of the tail of these rats at the time of inoculation and 2 and 3 weeks later failed to show trypanosomes. Three weeks after inoculation, the rats were sacrificed and repeated centrifugation of blood samples and selection and recentrifugation of the buffy coat layer failed to yield trypanosomes. Stained smears from the ventricle of the heart and from the spleen were negative also. It would have been desirable to have inoculated younger rats as well, but these were not available.

TAXONOMY

Descriptions of species of trypanosomes in the past have been based primarily or entirely on the morphology of the adult. It has been shown by Davis (1952) most recently that this approach is frequently invalid for the trypanosomes of rodents and that other biological characteristics, such as site and method of multiplication are more important. Since most of the known species of trypanosomes occurring in rodents are known largely by the morphology of the adult, which is similar to that of the lemming trypanosome, it is impossible at this time to decide what species name should be applied to the trypanosome of the collared lemming. On the basis of the morphology of the adult trypanosome and the systematic position of the collared lemming, one would suspect a close relationship between the lemming trypanosome and *T. microti*, described from various species of *Microtus* in Europe and North America (Laveran and Pettit, 1909; Lavier, 1921; Coatney, 1935; Wood and Wood, 1937; Nelson, 1949) and *T. evotomys* described from an *Evotomys* (now *Clethrionomys*) by Watson and Hadwen (1912). The genera *Microtus*, *Clethrionomys*, and *Dicrostonyx* belong to the subfamily MICROTINAE and are represented in the North by species, some of which have overlapping geographic ranges. At Churchill, specimens of *Microtus pennsylvanicus*, a known host of *T. microti*, (Coatney, 1935), and *Dicrostonyx torquatus* were sometimes trapped in the same runways, however, as a rule *Dicrostonyx* occupied higher and rockier terrain than *Microtus*.

BIOLOGICAL EFFECTS OF TRYPANOSOMIASIS

Trypanosomiasis was not responsible for the death of any of the 110 collared lemmings under observation and the behavior of the infected lemmings appeared normal. This was true even for one animal (#34) that was kept at 18° F. for over one month. Specimen #11 had apparently been infected before capture at Churchill. Shortly after capture, this female lemming produced a litter of at least 5 young, a normal number for this species. The blood of one of these young (#61) was examined 6 months later and contained trypanosomes. However, substantial evidence is lacking regarding the effect of trypanosomiasis on the growth and reproductive potential of the lemming host.

Anemia was the only marked effect of trypanosomiasis. The intensity of the anemia appears to parallel the number of circulating trypanosomes (see Table I). In specimen #34, with the largest number of trypanosomes, the erythrocyte count was about one-half the normal count for lemmings maintained in the cold (range = 11.51×10^6 to 15.22×10^6/cmm.). The white blood cell count does not seem to have been affected.

From these observations one can conclude that natural trypanosomiasis has a weakening effect on the lemming host, and that it is probably only a rare cause of death in these animals.

Since the collared lemming is such a widespread and basically important member of the arctic fauna, further study of the life cycle, transmission and pathogenicity of the natural trypanosome of the lemming seems highly desirable.

SUMMARY

A trypanosome occurring naturally in the peripheral blood of the collared lemming (*Dicrostonyx torquatus*) is described as morphologically similar to the trypanosomes found in related hosts. Natural trypanosomiasis in the lemming was never a cause of death of the hosts studied. The only marked effect was anemia, which was in direct proportion to the number of circulating adult trypanosomes. In the animal with the most severe infection, 58,000 trypanosomes per cmm. of blood, the erythrocyte count was about one-half the normal value. Intensive search for immature and multiplicative forms of trypanosomes in organs of the host were unsuccessful. An attempt at producing an infection in adult Sprague-Dawley rats by subcutaneous injection of infected lemming blood was unsuccessful.

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OCCURRENCE AND DISTRIBUTION OF HYMENOLEPIDID CESTODES IN SHREWS

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A survey of the literature dealing with cestodes of shrews reveals the increasing interest of contemporary parasitologists in this poorly known group of symbionts. In the United States approximately 18 species have been described from shrews in the last 15 years. These reports deal almost exclusively with morphology of the cestodes; however, information on distribution, host specificity, rate and intensity of infection, is either fragmentary or not available in published form.

Examination of autopsy data from a relatively large number of shrews, collected primarily by Rausch, enabled us to obtain some information on the subjects listed above. During the study of our material, it also became desirable to compare some of the data from our shrews with those obtained from shrews elsewhere and to extend this comparison to other vertebrate groups, specifically with reference to infection with hymenolepidid cestodes.

The present study is based on an examination of 452 shrews belonging to 11 species collected during the last 10 years in the United States and in Alaska. Numbers and kinds of cestodes found in each shrew were recorded; all the material obtained from each host was preserved.

We are indebted to several workers for their help in the collection of the shrews: Mrs. Betty Locker Pope, 914 E. 61st St., Chicago 37, Illinois; Mr. Everett Schiller, U. S. Public Health Service, Anchorage, Alaska; and Dr. Oliver P. Pearson, Museum of Vertebrate Zoology, University of California, Berkeley.

HOST DISTRIBUTION OF THE SPECIES OF *HYMENOLEPIS* IN VERTEBRATES

At present, the genus *Hymenolepis* Weinland, 1858, contains approximately 400 species. The majority of these occur in birds and only a relatively small number parasitize mammals. Over 100 species have been reported from ANSERIFORMES, about 44 species from CHARADRIIFORMES, 36 from PASSERIFORMES, 18 from CICONIIFORMES, 16 each from GALLIFORMES and GRUIFORMES, 10 from COLUMBIFORMES, 8 from PELECANIFORMES and three or less from the remaining orders of birds (Hughes, 1940). Judging from the available host lists, host specificity of avian hymenolepidids is relatively low. *Hymenolepis coronula* (Dujardin, 1845) is known to parasitize 10 species of ducks, and *H. serpentulus* (Schrank, 1788) 25 species of passeriforms. Others may occur in hosts belonging to as many as three orders of birds. However, with the exception of *H. lanceolata* (Bloch, 1782), the species which parasitize birds have not been found in mammals.

The species of *Hymenolepis* in mammals differ considerably from those in birds

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in their distributional pattern. Not only is the number of species reported from mammals much smaller (approximately 90, as compared to several hundred in birds), but there is also less overlap in occurrence among different orders of mammals. The only known ordinal overlap is that of *H. nana* (von Siebold, 1852) and *H. diminuta* (Rudolphi, 1819), which occur in both rodents and primates. Of the ninety or so species of *Hymenolepis* parasitizing mammals, 4 only have been reported from primates, about 25 from rodents, 8 from chiropterans, 1 from marsupials, 1 from carnivores, and about 50 from insectivores. None has been reported from lagomorphs, ungulates, or herbivores generally, or from the marine mammals. From the foregoing it is evident that in mammals the genus *Hymenolepis* has become adapted most successfully to species of INSECTIVORA: these are parasitized by a number of species larger than the combined total number found in all other mammalian groups. This relationship is even more obvious when one considers that the living genera of rodents (with 25 species of *Hymenolepis*) number 344, and the living genera of insectivores only 71 (Simpson, 1945). Furthermore, it is probable that rodents have been more thoroughly investigated than have insectivores, primarily because insectivores are more limited in distribution and caught with relative difficulty (Pearson, 1954). Also, because of their small size, many of the internal parasites of insectivores, including hymenolepidids, probably still await recognition and description.

Within the order INSECTIVORA, the shrews are hosts to more than 30 of the 50 or so hymenolepidids described to date. Sixteen of these, including 2 species of the genus *Protogynella* Jones, and *Diorchis reynoldsi* Jones (see Schiller, 1953), were described from shrews in North America. Although no thorough comparative study has been made, the available evidence suggests that the North American species are distinct from European ones. From available North American and European data it is also apparent that the species which parasitize shrews are not found in families other than the SORICIDAE, although it will be seen that host specificity does not extend to the different soricid species.

HYMENOLEPIDID CESTODES IN SHREWS

Before attempting to analyze the hymenolepidid cestodes of shrews in greater detail, it should be mentioned that the cestode fauna of these mammals is with few exceptions limited to members of the HYMENOLEPIDIDAE. The only non-hymenolepidid cestodes described from North American shrews are the dilepidid, *Liga soricis* Neiland, and the linstowiid, *Oochoristica pennsylvanica* Chandler. *Choanotaenia* sp. has been found in shrews in Alaska. The only non-hymenolepidid cestodes described from European shrews are two species of the dilepidid genus *Choanotaenia*. Recently (Soltys, 1953) a species of *Dicranotaenia* (syn. *Hymenolepis*) was described from shrews in Poland. This lack of variety in cestode parasites is somewhat surprising and is reminiscent of the situation in ruminants and horses, in which the adult cestode fauna is essentially limited to different anoplocephaline genera.

Among the hymenolepidid cestodes of shrews, one observes certain indications of trends in morphological patterns and specializations. The most obvious one is very small body-size, which, in a general way, can be correlated with the small size of the hosts. The mature strobila in some of the species is less than one-half millimeter long. In the largest species, the strobila attains a length of 100 mm. (*H.*

anthocephalus Van Gundy). In 11 European species, strobilar length is not more than 10 mm. The longest European specimen known to us (Rausch, unpublished) measures over 50 mm. Most of the hymenolepidids described from non-soricid insectivores (moles, hedgehogs) are considerably larger. This does not mean that small tapeworms, or specifically small hymenolepidids, always occur in relatively small hosts, nor is it to be understood that relatively large tapeworms never occur in small host species. It is desirable, however, to emphasize that shrews as a group are the smallest mammals and that they harbor a relatively large number of the smallest tapeworms described.

The shape of the strobila is distinctive and even diagnostic for some of the very small hymenolepidids (Plate 1). Terminal gravid proglottids may differ markedly in size and shape from preceding proglottids, so that the transition between gravid and other parts of the strobila may be very abrupt. In addition, the relatively small number of proglottids as well as the structure of the cirrus or vagina, may contribute to distinctiveness in shape of the strobila in different species. In *H. virilis* Voge, for example, the cirrus is proportionately very large and bears relatively few, but unusually long and slender spines. In *H. sphenomorphus* Locker and Rausch, the terminal portion of the cirrus is enlarged, giving it a club-shaped appearance; in *H. intricatus* Locker and Rausch, the terminal portion of the vagina is highly modi-

TABLE I.—*Incidence of tapeworm infection in shrews in California*

Host species	Number examined	Number infected	Number uninfected
Sorex bendirei	4	4	0
S. pacificus	15	10	5
S. vagrans	19	13	6
S. ornatus	2	2	0
S. trowbridgei	5	5	0
S. palustris	2	2	0

fied. With the exception of the scolex of *Protogynella*, the scolices in American shrew hymenolepidids do not have any unusual features.

Examination of North American data on incidence, intensity of infection and multiple infection with hymenolepidid cestodes, shows that shrews are frequently, as well as heavily, parasitized animals. Of 138 *Blarina brevicauda* examined by one of us (Rausch) from 1943–1948 in Ohio, Michigan and Wisconsin, 52 (or 37%) were infected with tapeworms which, with one single exception, belong to the HYMENOLEPIDIDAE. Similarly, of 190 *Sorex cinereus* examined during 1947–1948 in Wisconsin, 91 (or 48%) were infected with hymenolepidids. No other tapeworms were found in this host. Of 47 shrews from California examined by Voge, 36 (or 77%) were infected with tapeworms. Only two of these hosts (*Sorex bendirei*) were infected with cestodes other than hymenolepidids (Table 1).

Of 50 *Sorex vagrans* collected by Betty Locker Pope in Oregon and Washington and examined by Rausch, 47 or 94% were infected with cestodes. Except for unidentified specimens from 7 of these hosts, all cestodes were hymenolepidids (*Hymenolepis* and *Protogynella*).

Intensity of infection varies considerably in different host individuals and seems to be correlated with general body-size of the tapeworm species involved. From our data, it appears that relatively large tapeworm species occur in smaller numbers and very small species in larger numbers, per host individual. Intensity of infection with *Hymenolepis anthocephalus* Van Gundy, and *Hymenolepis falculata* Rausch and Kuns is illustrated in Tables II and III.

TABLE II.—Hymenolepis anthocephalus *in* Blarina brevicauda

Number of worms	1	2	3	4	5	6	10	11	15	17*	21*	48*
Number of hosts	9	5	4	1	6	2	1	1	1	1	1	1

* Immature worms.

It is obvious that 1–5 specimens per host individual occur much more frequently than larger numbers of worms. It should be pointed out that no other cestodes were present in the hosts listed in Tables II and III. A similar numerical relationship was observed for *Hymenolepis horrida* (von Linstow) in the mouse *Peromyscus californicus* (see Voge, 1952). However, species of smaller body size tend to occur more frequently in large numbers. This is seen in Table IV which illustrates the distribution of *Hymenolepis parva* Rausch and Kuns, in *Sorex cinereus.*

Strobilar length in *H. parva* is about 5 mm. Strobilar lengths of *H. falculata* and *H. anthocephalus* are about 40 mm. and 100 mm., respectively. The few data available on intensity of infection with very small hymenolepidids measuring 2 mm. or less, indicate a tendency toward even higher numbers of specimens present in individual hosts. Specimen numbers for *Protogynella* in *Sorex cinereus* are as follows: 13, 87, 95, 245 (Rausch); in *Sorex vagrans:* 1, 1, 1, 3, 3, 4, 5, 5, 6, 7, 8, 10, 15, 30, 30, 35, 40, 50, although in this series, the majority of the hosts were infected

TABLE III.—Hymenolepis falculata *in* Sorex cinereus

Number of worms	1	2	3	4	5	6	9	13*
Number of hosts	10	7	4	2	2	1	1	1

* Immature worms.

with one or more species of *Hymenolepis* in addition to *Protogynella;* in *Blarina brevicauda:* 29, 260 (Oswald, 1955); in *Sorex trowbridgei:* 13, 39 (Voge).

Multiple infections with hymenolepidids, although relatively infrequent in the shrews examined from the Middle West, are commonly seen in shrews on the Pacific Coast. Of 21 shrews examined in California (Voge), 6 were infected with one hymenolepidid species, 9 with 2 species, 4 with 3 species and 2 with 4 species, so that 15 of the 21 shrews contained more than one species of hymenolepidids. Neiland (1953) reported 7 different cestode species from one *Sorex bendirei* in Oregon. Of 40 *Sorex vagrans* examined by Pope from various localities in Oregon and Washington, 11 were infected with one hymenolepidid species, 16 with 2 species, 12 with 3 species, and 1 with 4 species, giving a total of 29 of 40 host individuals infected with more than 1 species of hymenolepidids. This high incidence of multiple infections may be in part attributable to the fact that most of the North American species described from shrews do occur on the Pacific Coast. However, for many of the Eastern and Midwestern shrews no parasitological information is available at present.

Our records indicate a complete lack of host specificity of hymenolepidids in different species of shrews. *Hymenolepis parva* has been found in 7 species of

TABLE IV.—Hymenolepis parva *in* Sorex cinereus

Number of worms	1	2	3	4	5	7	9	10	11	12	15	18	20	25	30	39	50
Number of hosts	7	3	1	1	3	1	1	1	1	3	2	1	2	2	1	1	2

shrews, *H. falculata* in 5 species, *H. kenki* and *H. macyi* each in 4 species. With regard to geographic distribution, the available data indicate that some of these hymenolepidids occur in California, Oregon and Alaska (*H. sphenomorphus*), others (*H. parva*) in the Eastern and Western United States as well as in Alaska. New host records from Alaska are presented in Table V.

TABLE V.—*Records of hymenolepis from shrews in Alaska*

Sorex	Hymenolepis	Locality
S. obscurus	*H. parva*	Anaktuvuk Pass
	H. falculata	2 mi. S. Anchorage
	H. sphenomorphus	8 mi. S. Anchorage
	H. intricatus	6 mi. N. Anchorage
S. cinereus	*H. parva*	Nunivak Island
	H. falculata	2 mi. S. Anchorage
	H. schilleri	Katmai National Monument
	H. sphenomorphus	8 mi. S. Anchorage
S. palustris	*H. parva*	20 mi. NE. Anchorage

Additional records of hymenolepidids in Alaskan shrews were reported by Johnson and Clanton (1954) and Voge (1955). Thus it appears that the distribution of these cestodes is by no means restricted to small areas, and that their occurrence is probably limited only by the distribution of the definitive hosts and their intermediate hosts, although nothing definite is known about the latter. None of the hymenolepidids from shrews has been reported from hosts other than shrews.

DISCUSSION

In his classification of mammals, Simpson (1945, p. 175) made the following statement: "It is probable that the most characteristic insectivores, such as the erinaceoids and the soricoids, do belong to an order of unified origin." With this idea in mind, it is of interest to review the data of our parasitological investigations of these groups of insectivores. We have stated that the family SORICIDAE, in contrast to the TALPIDAE and ERINACEIDAE, harbors a morphologically distinct cestode fauna which is apparently restricted to the SORICIDAE. The TALPIDAE and ERINACEIDAE, on the contrary, harbor forms which, morphologically at least, conform to a common pattern, observed in non-soricid insectivores as well as in other mammals. One may infer from this observation that physiologically and ecologically at least, the SORICIDAE have diverged and specialized much more than have the TALPIDAE and ERINACEIDAE.

Such specializations would have to provide suitable ecological and physiological environments for the successful completion of life cycles of the cestode parasites in order to permit speciation within this group. It is apparent from the data presented in this study, that extensive speciation has occurred within the hymenolepidids of shrews. Therefore, a discussion of pertinent characteristics of the shrew hosts may be of interest. The first characteristic to be considered is food habits. It is known that many hymenolepidid cestodes of terrestrial vertebrates require an insect or other invertebrate intermediate host. Of the European hymenolepidids from shrews, *H. furcata* (Stieda, 1862) uses a beetle, and *H. pistillum* (Dujardin, 1843) a millipede (Joyeux and Baer, 1936). Analyses of contents of shrew stomachs (Johnson and Clanton, 1954) revealed a large number of insects belonging to several different orders, as well as spiders.

However, the insectivorous habit alone does not explain the high incidence of

infection, the frequent multiple infections, nor the relatively large number of species present in shrews generally. An explanation of some of these facts can probably be found in the extreme voraciousness of shrews (Pearson, 1954). The hazards involved in the successful completion of a cestode life cycle (viability and survival of eggs, availability and ingestion of infected intermediate hosts, infectivity of larval stages, etc.) are many and the loss of eggs and larval stages is bound to be extensive. A high infection rate as well as frequent multiple infection of the definitive host must reflect a relatively high infection rate of the intermediate hosts which are eaten in large numbers. The voraciousness of shrews may well be an important factor determining the high incidence of infection with hymenolepidids. The frequency of multiple infections with hymenolepidids may be in part explained by the variety of insects and other invertebrates consumed and by a minimal or non-existing cross immunity of the definitive host.

The most controversial question is that of speciation, particularly the external factors predisposing to speciation within the HYMENOLEPIDIDAE. As stated previously, among the mammals the INSECTIVORA and the shrews in particular harbor the largest number of species, while among the birds, the ANSERIFORMES harbor the largest number. The assumption that extensive speciation of the host group might be followed by extensive speciation of their parasitic fauna is possibly a reasonable one. However for a relatively homogeneous group such as the HYMENOLEPIDIDAE, this does not hold. Every group of parasites has specific requirements which are intimately associated with the ecology of their definitive as well as their intermediate hosts. A vertebrate group, no matter how numerous (in terms of genera and/or species) is a suitable host for any given type of parasite only if it has the ecological and physiological specializations essential for the completion of the life cycle of the parasite. One example is that of anseriform and falconiform birds with approximately 64 and 89 genera respectively. While there are over 100 species of *Hymenolepis* described from ANSERIFORMES, only two have been reported from FALCONIFORMES, one of these two species being a frequent parasite of passeriform birds, *H. passeris* (Gmelin, 1790), and probably accidentally ingested by the hawk (*Accipiter nisus*). As far as known, the intermediate hosts of *Hymenolepis* spp. in anseriforms are various crustaceans, *H. collaris* (Batsch, 1786), *H. coronula* (Dujardin, 1845), and molluscs, *H. microsoma* (Creplin, 1829). Adaptation to the food habits of this group of birds is obvious.

Extensive speciation of hymenolepidids in shrews must be in part related to food habits, both qualitative and quantitative; assuming that the amount of insects consumed is proportional to the infection rate and survival of the hymenolepidid population, there may be a greater opportunity for the survival of mutants when the infection rate is high. It is likely that a relatively dense and stable host population which maintains a constantly high infection rate over long periods of time is an important condition for speciation of the parasitic fauna of this host population.

The paucity of cestodes other than hymenolepidids in shrews cannot be explained satisfactorily on the basis of food habits. One might expect better representation of davaineids, linstowiids and dilepidids, members of which are known to employ insect or other invertebrate intermediate hosts.

The frequency of numbers by which certain species are represented in their hosts, deserves comment. Li and Hsü (1951) reported that "the most frequent

numbers of worms in their naturally infected hosts are the lowest numbers, especially of one or two worms." Their findings are in accord with the data on shrew hymenolepidids presented in this study, at least with respect to species of relatively large body-size. The species of very small body-size, however, are more often represented by larger numbers. This phenomenon may well be related to the total amount of usable intestinal space which is available to a given tapeworm species, and which provides the necessary physiological niches as well as sufficient food for the individuals of this tapeworm species. Thus the amount of living adult tapeworm tissue in any vertebrate host is limited by the intestinal environment provided by the host; in any given situation, more living space is probably available for large numbers of small individuals than for large numbers of large individuals, as illustrated to some extent by experiments on the "crowding effect" (Read, 1951). Unfortunately, little is known about differences in specific environmental requirements of different species of hymenolepidid tapeworms.

SUMMARY AND CONCLUSION

From the evidence presented in this report, certain conclusions may be reached. Among the mammals, hymenolepidid cestodes have attained their greatest development in the soricid insectivores which harbor more species than any other group of mammals. The cestode fauna of soricid insectivores is, with few exceptions, limited to the family HYMENOLEPIDIDAE.

The hymenolepidid species in shrews are morphologically distinct from hymenolepidids in non-soricid insectivores or in other mammals. They seem to be much more host-group specific than are other hymenolepidids. The species occurring in shrews have not been found in non-soricid hosts. Conversely, hymenolepidids described from non-soricid mammals have not been reported from shrews. From the evidence available, it may be concluded that host specificity of hymenolepidids of insectivores and particularly of shrews is much more pronounced than that of other hymenolepidids.

Hymenolepidid species described from North American shrews do not occur in Europe, and the European species do not occur in America. However, several of the hymenolepidid species from North American shrews have a wide distribution which includes various areas of the United States and Alaska (see new host records presented above).

The high infection rate as well as the high incidence of multiple infections with hymenolepidid cestodes in shrews is largely a reflection of the food habits of the hosts. The nature of the food as well as the quantity consumed play an important role in the rate as well as the intensity of infection. From the high incidence of multiple infections it may be concluded that cross immunity of the shrew host is relatively low and that the different hymenolepidid species have achieved a high level of physiological compatibility.

Hymenolepidid species of relatively large body size occur most frequently in low numbers in individual shrew hosts; species of very small body size are frequently present in large numbers.

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EXPLANATION OF FIGURES

All drawings made with the aid of a camera lucida.

FIG. 1. *Hymenolepis virilis* Voge, 1955.

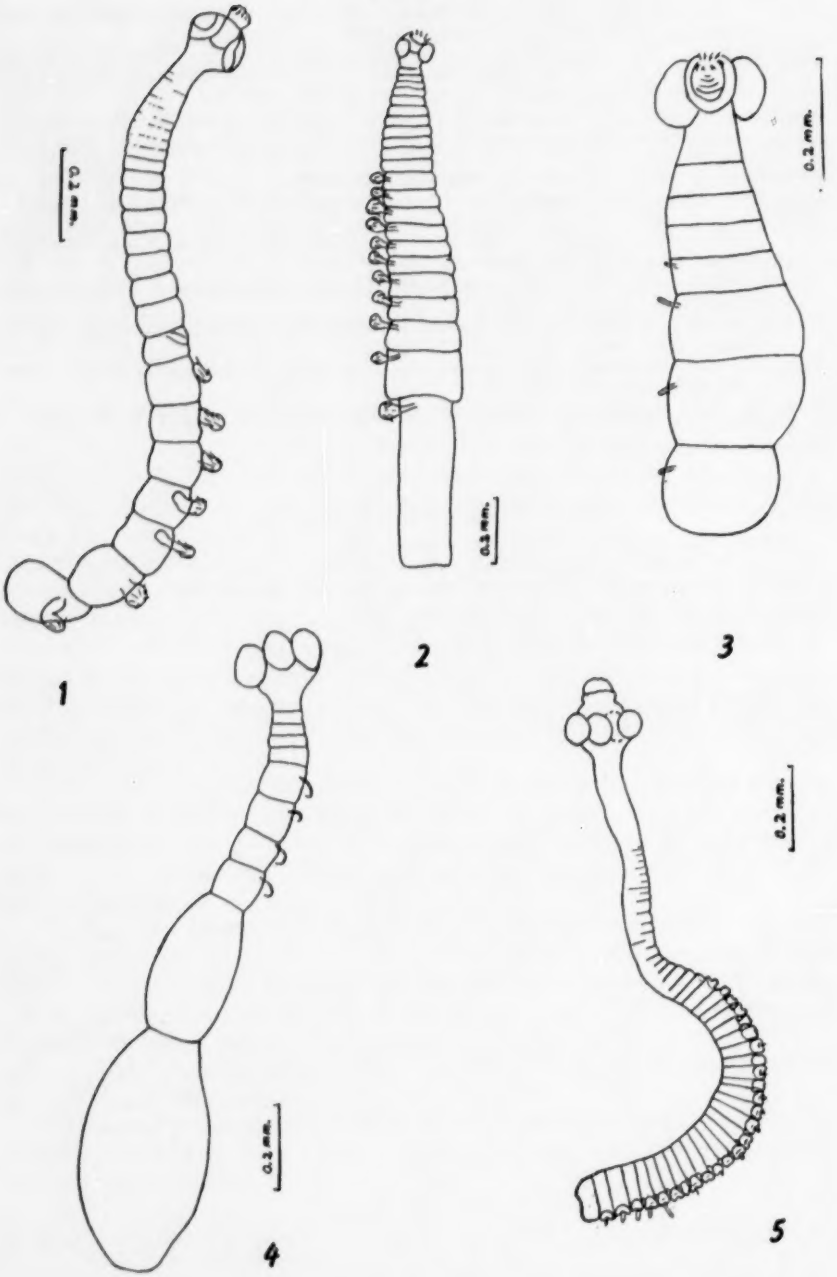
FIG. 2. *H. sphenomorphus* Locker and Rausch, 1952.

FIG. 3. *H. parvissima* Voge, 1953.

FIG. 4. *H. macyi* Locker and Rausch, 1952.

FIG. 5. *H. intricatus* Locker and Rausch, 1952.

PLATE I



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THE EFFECTIVENESS OF PIPERAZINE CITRATE AGAINST INTESTINAL HELMINTHS OF THE CAT AND DOG[1]

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Piperazine salts such as the citrate[3] are now recognized therapeutic agents for use in human infections with the pinworm *Enterobius vermicularis,* (Mouriquand, Roman and Coisnard, 1951; Turpin, Cavier and Savaton-Pillet, 1952; White and Standen, 1953; Brown, Chan and Hussey, 1954; Bumbalo, Gustina and Oleksiak, 1954) and the roundworm *Ascaris lumbricoides,* (Fayard, 1949; Mouriquand *et al,* 1951; Brown and Sterman, 1954; Goodwin and Standen, 1954). Various piperazine salts have also been found to be effective against *Syphacia obvelata, Aspiculuris tetraptera,* (Mouriquand *et al,* 1951; Standen, 1953; Brown, Chan and Hussey, 1954) and *Trichinella spiralis,* (Chan and Brown, 1954) in the mouse, and against some of the intestinal parasitic helminths of a variety of domestic and wild animals (Mouriquand *et al,* 1951; Sloan, Kingsbury and Jolly, 1954).

We wish to report the results of experiments in which piperazine citrate was fed to cats and dogs naturally infected with a variety of intestinal helminths.

MATERIALS AND METHODS

The animals used in this study were selected by a fecal examination for helminth ova (saturated saline centrifugation) and for tapeworm segments. The piperazine citrate was given *per os* usually before feeding, at a dosage level arbitrarily set at 100 mg./kg. (hexahydrate equivalent, see footnote 4) of the animal's body weight given in a single daily dose for ten successive days. The cats were dosed by placing each in a box which fitted closely around the animal's neck, leaving the head exposed, and administering the drug, enclosed in a gelatin capsule, by means of a curved hemostat. The dogs were given a flavored piperazine citrate syrup[4] by ejecting the calculated volume from a syringe into the cheek pouch of each animal. The syrup was accepted readily. The animals were maintained on a stock diet and kept in individual metabolism cages, on wire mesh, throughout treatment. A number of infected cats served as untreated controls, in order to determine the number of hookworms or tapeworms passed spontaneously. These animals were subsequently given a course of treatment for 10 consecutive days. Feces passed during and for one day following treatment were collected and washed through a fine mesh sieve, and a careful search made for expelled worms. The animals were sacrificed 24 hours after the completion of therapy and their entire gastrointestinal

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[1] Acknowledgment is made to Dr. H. W. Brown, School of Public Health, Columbia University for valuable consultation during this study.
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[3] Syrup and tablets were supplied under the trade name 'ANTEPAR' citrate brand piperazine citrate by Burroughs Wellcome and Co. (U.S.A.) Inc. Tuckahoe, N. Y.
[4] Dosage of different piperazine salts is usually given in weight equivalents of hexahydrate, a practice continued here. Piperazine base equivalents would be a more fundamental unit for comparisons, but the voluminous early literature was reported in hexahydrate dosages. (For a review of this early work, cf. Hanzlik, 1917).

tract was thoroughly searched for adult helminths to ascertain the effectiveness of the drug.

RESULTS AND DISCUSSION

Of the cats examined, 31 harbored helminths. Eleven had ascarids, *Toxocara cati* and/or *Toxascaris leonina*. Nine had *Dipylidium* sp. Twenty harbored hookworms, *Ancylostoma* sp. *Taenia taeniaeformis* was found in seven. Thirteen dogs harbored helminths. Two had ascarids, *Toxascaris leonina;* 9 had *Dipylidium caninum;* 3 contained hookworms, *Ancylostoma caninum;* a *Taenia pisiformis* was found in one animal; and nine dogs had whipworms, *Trichuris vulpis*.

As shown in Table I, piperazine citrate was remarkably effective against ascarids.

TABLE I.—*The effects of piperazine citrate on ascarids*

Animal	No. of worms eliminated during treatment day indicated*										Total eliminated	Found at autopsy
	1	2	3	4	5	6	7	8	9	10		
Cat	3										3	0
Cat											0	1 (dead)
Cat	9										9	0
Cat		1									1	0
Cat		2									2	0
Cat		4									4	0
Cat	1										1	0
Cat	2										2	0
Cat		2	2								4	0
Cat		7	2								9	0
Cat	2	1									3	1
Dog				4							4	0
Dog	1	1									2	0

* Treatment days are counted as starting at the time of the first dose of drug.

The elimination of these worms began promptly and, with the exception of 2 worms, all 46 observed in the collecting pans were passed within 4 days after therapy was begun. One of the two ascarids found in the intestine at autopsy was still alive, but the other apparently was dead.

Piperazine citrate was slightly effective against the hookworm, *Ancylostoma caninum*. After 10 days of treatment with piperazine citrate 29 worms (18% of total present) had been eliminated from 11 of the 20 infected cats, but 131 worms

TABLE II.—*Hookworms eliminated after piperazine citrate*

Cat No.	Daily dose mg.	No. of worms eliminated during treatment day indicated										Total eliminated	Found at autopsy
		1	2	3	4	5	6	7	8	9	10		
						Before Treatment							
62	0											0	—
63	0											0	—
71	0											0	—
66	0											0	—
101	0											0	—
102	0											0	—
109	0											0	—
113	0											0	—
						After Treatment							
62	300	1					1					2	12
63	250	6				1						7	7
71	250											0	5
66	350											0	7
101	300			3								3	18
102	300	1	1	1				1				4	9
109	250						1					1	7
113	250											0	9
Dog No.													
24	900											0	4
34	1500											0	5
39	700	42	3	2			1					48	240

remained and were observed at autopsy. During treatment, 48 hookworms had been passed by the most heavily infected of three dogs, but 249 worms were found at autopsy. No cat or dog was completely cleared. An inspection of the typical results given in Table II shows that the elimination of hookworms in the cats was apparently due to the treatment with piperazine citrate, since not one of the 8 cats passed a single hookworm during a 10 day observation period, yet these same cats passed 17 worms during the treatment period.

During the 10 day piperazine citrate treatment 4 *Taenia*, complete with scolex, were passed by 3 of the 7 *Taenia*-infected cats. On autopsy, 15 worms were found within the animals. Two untreated control *Taenia*-infected cats lost none of their worms during a comparable 10 day period. A single infected dog failed to eliminate its one *Taenia pisiformis* during treatment.

Piperazine citrate failed to cause the elimination of any of the tapeworms *Dipylidium caninum* in either the cats or dogs studied. Likewise piperazine citrate was apparently without effect on the whipworm *Trichuris vulpis* in dogs.

Toxic manifestations were not observed in the treated animals, some of which were in various stages of pregnancy, and no gross pathological lesions of their gastrointestinal tract were noted at autopsy. Recently, Bąsnuevo (1954) reported that the course of pregnancy is not adversely affected following the administration of diethylenediamine (piperazine) hydrate to women infected with pinworms.

Thus, the results reported here indicate that piperazine citrate in the dosage employed is very effective against dog and cat ascarids, is without observable effect against *Dipylidium* and dog whipworms, and is somewhat effective against both *Taenia* and hookworms. It is possible that increased dosage of the drug might have led to greater effectiveness against these last two species. At the least, no adverse effects would be anticipated from the use of this drug to clear *Ascaris* from multiple infections, and some drop in the hookworm and/or tapeworm burden might occur.

SUMMARY

Piperazine citrate was administered orally to dogs and cats at a rate of 100 mg./kg. daily for 10 consecutive days. This treatment was extremely effective against ascarids, somewhat effective against *Ancylostoma caninum* and *Taenia taeniaeformis*, but ineffective against *Trichuris vulpis* and *Dipylidium caninum*.

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STUDIES ON THE HELMINTH FAUNA OF ALASKA. XXVI. SOME OBSERVATIONS ON THE COLD-RESISTANCE OF EGGS OF *ECHINOCOCCUS SIBIRICENSIS* RAUSCH AND SCHILLER, 1954

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Alveolar hydatidosis, caused by the larval stage of the cestode *Echinococcus sibiricensis* Rausch and Schiller, 1954, is an important parasitic disease of man on St. Lawrence Island, Alaska. The adult parasite is harbored by sledge-dogs and arctic foxes, which disseminate the infectious eggs. Inasmuch as these eggs, upon elimination from the definitive host, are subjected to temperatures below freezing for much of the year, knowledge of the effect of such conditions on their infectivity is important for a better understanding of the epidemiology of this disease. The following observations have therefore been made on the basis of experimental studies in the laboratory.

A preliminary experiment was conducted using eggs of *E. sibiricensis* obtained at the routine autopsy of a series of foxes, *Alopex lagopus* L., collected on St. Lawrence Island during December, 1953. These carcasses had been frozen by exposure to outdoor temperatures prior to shipment from the Island and, during a delay en route at Nome, Alaska, had been exposed to temperatures as low as – 40° C. After arrival at Anchorage, the carcasses were stored outside the laboratory for 6 weeks before the examinations were undertaken. During this period the temperature ranged from 5° C. to – 37° C.[1] At the time of the autopsies, the carcasses were allowed to thaw for about 12 hours at room temperature. Gravid proglottids of *E. sibiricensis*, obtained from each of 3 carcasses, were fed separately to 3 field voles, *Microtus pennsylvanicus* (Ord). Examination of these voles at the end of 2 weeks disclosed that all harbored alveolar larvae of *E. sibiricensis*. In view of this evidence that eggs of *E. sibiricensis* can survive prolonged exposure to variable and extreme cold, experimental studies were subsequently conducted under known conditions of temperature and exposure-time.

In the first experimental study described below, the temperature was kept constant, but the time of exposure was varied. For this purpose, large numbers of strobilae of *E. sibiricensis* were collected at the autopsy of an experimentally infected arctic fox. Some of the natural semi-fluid content of the small intestine, containing a heavy concentration of these cestodes, was placed in each of 80 1.5 cc.

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[1] U. S. D. C. Weather Bureau Climatological Data, Alaska. 1954.

glass vials. These vials were fitted with cotton stoppers and placed in a mechanical freezer which was kept at a constant temperature of −26° C. Freezing occurred within 2 hours. At intervals of 24 hours, a vial was removed from the freezer, allowed to thaw at room temperature and equal amounts of the contents were fed by means of a stomach tube to each of 3 red-backed voles, *Clethrionomys rutilus dawsoni* (Merriam).[2] After the 15th day, the interval of time was increased so that the rest of the feedings occurred on the 35th, 54th, and 65th days from the time of placing the material in the freezer. Except for selected animals kept over longer periods of time for observations on larval development, all of the voles which had been given proglottids of *E. sibiricensis* were killed after 10 days and examined for the presence of alveolar larvae. The results of this experiment are summarized in Table I.

TABLE I.—*Results obtained upon feeding to red-backed voles the gravid proglottids of* E. sibiricensis *from semi-fluid intestinal contents of arctic fox, after exposure to constant temperature of −26° C.*

Vial number	Hours frozen	Number of animals infected	Date of infection	Date of examination	Results at autopsy
1	21	3	29 Sept., 1954	6 Oct. 1954	3 +
2	24	3	29 Sept.	7 Oct.	3 +
3	48	3	30 Sept.	8 Oct.	3 +
4	72	3	1 Oct.	9 Oct.	3 +
5	96	3	2 Oct.	10 Oct.	3 +
6	120	3	3 Oct.	11 Oct.	3 +
7	144	3	4 Oct.	(1) 12 Oct (2) 22 Oct.	3 +
8	168	3	5 Oct.	(1) 13 Oct. (2) 23 Oct.	3 +
9	192	3	6 Oct.	(1) 14 Oct. (1) 22 Nov.* (1) 28 Nov.*	3 +
10	216	3	7 Oct.	(1) 15 Oct. (2) 25 Oct.	3 +
11	240	3	8 Oct.	16 Oct.	3 +
12	264	3	9 Oct.	17 Oct.	3 +**
13	288	1	10 Oct.	18 Oct.	1 +
14	312	3	11 Oct.	19 Oct.	3 +**
15	336	3	12 Oct.	20 Oct.	3 +
16	360	3	13 Oct.	21 Oct.	3 +
17	35 days	3	7 Nov.	20 Nov.	1 + (light) 2 −
18	54 days	3	26 Nov.	7 Dec.	1 + (light) 2 −
19***	65 days	3	7 Dec.	17 Dec.	3−

* Animal died—extremely heavy infection.
** Infections ranged heavy, medium, light.
*** Microscopic examination showed most eggs ruptured.

Some of the original frozen material was used in a concurrent experiment, as follows: After exposure to a temperature of −26° C. for 382 hours, 8 vials were removed from the freezer and placed immediately in a Bowser Special Temperature Test Chamber (having a range of 30° C. to −60° C.) which had been previously standardized at a temperature of −31° C. A recording potentiometer was used during this operation to detect any deviation from the desired temperature. After 24 hours, the first vial was removed, allowed to thaw at room temperature, and equal amounts of the contents were administered to each of 3 red-backed voles. Upon removal of the vial from the chamber, the temperature of the chamber was immediately lowered 5° C. This procedure was repeated each day for the next 5 days until a minimum temperature of −56° C. had been reached. The material

[2] These were live-trapped near Anchorage, Alaska; all controls were negative. *E. sibiricensis* is not known to occur on the mainland of Alaska.

contained in the 2 remaining vials was utilized for purposes of microscopic examination to determine the morphological condition of the eggs after exposure to these extreme temperatures. *Post mortem* examinations of the voles were made at the appropriate time in the usual manner. The results of this experiment are summarized in Table II.

TABLE II.—*Results obtained upon feeding to red-backed voles the gravid proglottids of* E. sibiricensis *from natural semi-fluid intestinal contents of arctic fox, after exposure to extreme cold in the Bowser Special Temperature Test Chamber*

Temperature of chamber	Date and time temperature of chamber reduced 5° C.	Vial number	Date and time vial removed and contents fed to each of 3 voles	Hours of exposure to each temperature	Total hours of exposure in chamber	Results at autopsy
−31° C.	4:00 P.M. 14 Oct. 1954	1	4:00 P.M. 15 Oct. 1954	24	24	3+
−36° C.	4:00 P.M. 15 Oct.	2	4:00 P.M. 16 Oct.	24	48	1+ 2−
−41° C.	4:00 P.M. 16 Oct.	3	4:00 P.M. 17 Oct.	24	72	1+ 2−
−46° C.	4:00 P.M. 17 Oct.	4	4:00 P.M. 18 Oct.	24	96	3−
−51° C.	4:00 P.M. 18 Oct.	5	4:00 P.M. 19 Oct.	24	120	1+ 2−
−56° C.	4:00 P.M. 19 Oct.	6	4:00 P.M. 20 Oct.	24	144	3−

The final aspect of the study reported herein was concerned with the effect of prolonged exposure to low temperature on the viability of eggs of *E. sibiricensis* contained within the carcasses of naturally infected foxes. Twelve of a series of arctic foxes killed on St. Lawrence Island about December 1, 1954, and brought to Anchorage in a naturally frozen condition (temperatures on St. Lawrence Island had reached a minimum of −21° C.) were put into a mechanical freezer and maintained at a uniform temperature of −26° C. Periodically a carcass was removed, allowed to thaw for about 12 hours at room temperature, and examined for cestodes. Gravid proglottids of *E. sibiricensis* obtained from the infected carcasses were fed to red-backed voles. *Post mortem* examinations of the voles were made in the usual manner. The results are summarized in Table III.

TABLE III.—*Results obtained upon feeding to red-backed voles the gravid proglottids of* E. sibiricensis *after exposure, within the carcasses of arctic foxes, to temperature of −26° C.*

Carcass number	Date autopsied and eggs fed	Number of days in freezer at −26° C.	Number of of animals exposed	Results at autopsy
1	14 Dec., 1954	4	3	3+* (Heavily infected)
2, 3	6 Jan., 1955	27	6	6+ (Heavily infected)
4	21 Jan.	42	10**	7+ 2−
5	9 Feb.	61	3	3−
6	22 Mar.	102	3	3−

* One red-backed vole was killed 42 days after eggs were administered and the infected liver was fed to a dog.
** One animal died during the course of the experiment; no examination was made.

DISCUSSION

Dévé (1908) showed that eggs of *Taenia echinococcus* [syn. *Echinococcus granulosus* (Batsch, 1786)], kept in ordinary water at room temperature for 16 days, were infective to squirrels. He also infected squirrels using eggs which had been subjected to desiccation for 11 days. In a subsequent experiment Dévé (1910)

demonstrated that eggs of this species are resistant to freezing. He infected a suckling pig by feeding it pellets of fecal material containing eggs of *T. echinococcus* (syn. *E. granulosus*) which had been refrigerated (temperature maintained between −1 and +1 degrees) for 116 days. Bergstermann *et al.* (1951, p. 119) pointed out the high resistance of the eggs of *E. granulosus:* "Die Eier sind sehr widerstandsfähig gegen Austrocknen, Wassen und Kälte. Zwischen −1° und +1° bleiben sie über 100 Tage lebensfähig." Other studies on the viability of taeniid eggs, for the most part, have been conducted with *Taenia saginata* Goeze, 1782. Penfold *et al.* (1937) reported that eggs of this species, when stored in physiological saline solution at 2° to 5° C., were viable after 13.5 weeks, and that some eggs remained viable after being similarly stored for 9 weeks and then exposed on pastures to mild conditions in Australia for 8 weeks. Jepsen and Roth (1952) in Denmark

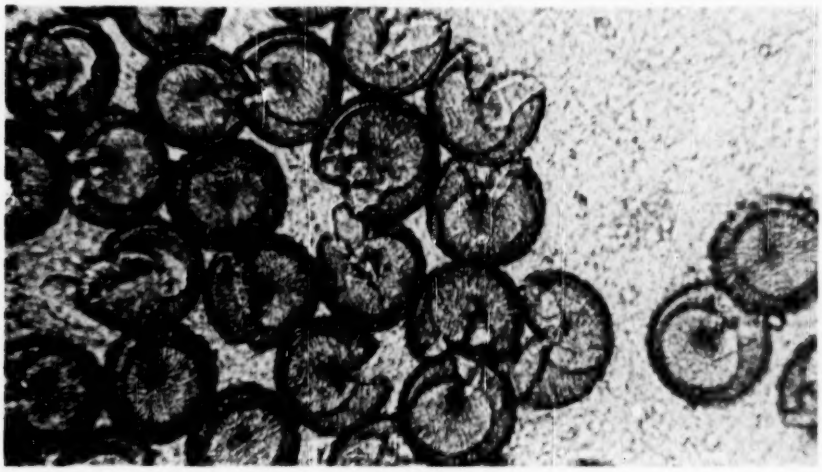

FIG. 1. Eggs of *E. sibiricensis* showing characteristic fracturing after exposure over period of 6 days to temperatures beginning at −31° C. and gradually reduced to −56° C.

found that a fairly large number of eggs of *T. saginata,* which had been exposed on grass to the open air from February to July, were infective to young calves. They also found that desiccation during exposure on grass in summer appeared to be important in limiting their ability to survive. On the basis of the results obtained by them, together with those of Penfold *et al.,* Jepsen and Roth (1952) stated "it seems quite probable that eggs of *T. saginata* may remain viable under natural conditions long enough to permit a protracted contamination of fields, water, and crops from contact with a human tapeworm carrier."

The results of the present study give evidence that eggs of *E. sibiricensis* are capable of withstanding sub-freezing temperatures without loss of infectivity. The fact that infections gradually became lighter and that negative results appeared among some of the test voles as the exposure time increased (e.g., at 35 and 54 days, Table 1) indicates that infectivity of the eggs decreases in inverse proportion to the length of time exposed and the extreme of low temperature to which they are subjected. After freezing, the duration of exposure seems to be more deleterious than the extreme of cold to which the eggs are exposed. It was possible

to infect a vole with eggs which had been subjected to a temperature as low as −51° C. However, microscopic examination of eggs (after thawing) which had undergone similar treatment in the temperature chamber disclosed that the shells of practically all were fractured (Fig. 1).

All infections in the test voles progressed normally as far as could be determined by comparison of the larvae with other larvae of the same age from experimental infections induced with eggs taken directly from the host and not exposed to cold. Further evidence of normal larval development was provided when cestodes matured in a dog fed the larval stage from a red-backed vole originally given eggs which had been subjected to freezing (from carcass #1, Table III).

It appears that a somewhat longer period of exposure (at −26° C.) is required to destroy the eggs of *E. sibiricensis* within the carcass of a fox than after their removal, indicating that some protection may be afforded by the carcass. This fact may be of significance in relation to winter survival of the eggs. Carcasses of foxes trapped by the Eskimo are usually fed to their dogs. In some years rabies outbreaks occur (see Rausch, 1953, p. 1168–1169) and large numbers of fox carcasses may be seen scattered over the tundra. Dead dogs are usually discarded near the villages. If eggs ingested by larval blowflies can be disseminated by the adult (see Schiller, 1954), survival of eggs in such carcasses may be of importance in the epidemiology of alveolar hydatidosis.

The feces of foxes and dogs deposited during the frost-free months (July and August) on St. Lawrence Island soon disintegrate and helminth eggs contained therein are more or less exposed to the elements. During the fall and winter eggs frozen *in situ* in feces are covered with snow. Although the air temperature may rarely go as low as −37° C., temperatures under a persistent cover of snow remain relatively high. Under these conditions it seems quite likely that eggs of *E. sibiricensis* remain infective throughout the winter. Studies under natural conditions on the survival of eggs of *E. sibiricensis* on St. Lawrence Island would be especially valuable.

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PARASITES OF MOUNTAIN SHEEP IN NEW MEXICO, WITH NEW HOST RECORDS*

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Parasites of big game animals are of interest not only because of their pathogenic potential for their wild hosts, but also because most of these pests are transmissible to domestic animals. A complete list of the species of parasites, particularly helminths, which domestic and wild ruminants have in common is too lengthy for inclusion in this paper. However, comparatively few species found in domestic ruminants are unreported from wild ruminants and *vice versa*. It is obvious, therefore, that where these groups intermingle on the range, the magnitude of parasitism in one may be profoundly influenced by its magnitude in the other (see: Longhurst and Douglas, 1953).

There are only a few reports of parasites from mountain sheep in the Southwest. The spinose ear tick, *Otobius megnini*, was found on this host in New Mexico by Kemper (1947) and Allen and Kennedy (1952). The latter authors, who examined only a single individual from the San Andres Refuge, also reported from it: *Dermacentor albipictus*, *Cysticercus tenuicollis*, *Nematodirus spathiger*, *Trichuris* sp., and *Skrjabinema* sp., the last named in large numbers. *Dermacentor hunteri* Bishopp, 1912, described from specimens collected near Quartzite, Arizona, is reported only from this host and only from a restricted area in Arizona (Bishopp and Trembley, 1945).

The writer has reexamined the pinworms identified as *Skrjabinema* sp. by Allen and Kennedy and tentatively regards them as *S. ovis* for reasons to be explained later.

The present study was made possible by the New Mexico Department of Game and Fish and thanks are due Mr. Paul Gordon, biologist in that department, for his assistance in arranging for the collection of material in the field. Serologic tests for brucellosis were made by Dr. R. T. McCarty of New Mexico College of A. and M. A. The writer is grateful for assistance received from several members of the staff of the Animal Disease and Parasite Research Branch, A.R.S.: Dr. John Lotze examined the blood smears; Mr. Allen McIntosh assisted with the identification of some of the ticks; Mr. Aaron Goldberg confirmed the identification of *Pseudostertagia bullosa;* Mr. John T. Tucker identified the whipworms tentatively and provided the comments which are given later concerning their identity; Mr. Kay Samson and Mr. Gerald Mayfield gave technical assistance in the laboratory.

MATERIALS AND METHODS

Post-mortem examinations for parasites were carried out on nine *Ovis canadensis mexicana*, taken during a special hunt in January, 1954, on Big Hatchet Mountain in southern New Mexico. This location is approximately on the Continental

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* This work was done at the State College, New Mexico, field station, in cooperation with the New Mexico Agricultural Experiment Station.

Divide, about 58 miles southwest of the city of Deming, and about 15 miles from the Mexican boundary. Most of the sheep were taken at elevations ranging from 4,000 to 8,000 feet.

Procedures used in the examinations were as follows: When the animal was killed, the viscera were removed by the hunter and placed in a cloth sack. At the same time, blood smears were made for parasitological examination and blood samples were taken for the detection of brucellosis. These materials were brought to the checking station with the carcass. There the carcasses were examined, first for external parasites. But, in some cases considerable time elapsed between the death of the animal and the examination for external parasites. Therefore, it is probable that some ticks may have become detached and the numbers given in this report may not be indicative of the true numbers present on the living animal. Circumstances prevented thorough examinations for gullet worms and examinations of the carotid arteries for *Elaeophora schneideri*. Hearts, lungs, and portions of the esophagus were examined. The rumen, abomasum, liver, and entire intestine were thoroughly examined. A fecal sample was removed from the rectum of each animal and preserved in formalin for detection of coccidial oocysts.

Abomasal and intestinal contents were washed into large containers and allowed to sediment, the supernatant then being poured off. This was repeated several times until the supernatant fluid was clear. An aliquot of the sediment, usually 10 percent, was then taken for microscopic examination for small species of nematodes. Total numbers of small worms were estimated from dilution counts. The remainder of each sample was examined for the larger parasites with the aid of 10- and 20-mesh screens in combination, the counts being made directly.

PARASITES FOUND

The kinds and numbers of parasites found and their incidence are shown in Table I. *Pseudostertagia bullosa*, which appears to be primarily a parasite of the

TABLE I. *Parasites found in 9* Bighorn Sheep in New Mexico*

Species	No. infected	% Positive	No. found
Dermacentor albipictus	4	44.4	1–6
Otobius megnini	2	22.2	3–5
Cysticercus tenuicollis	5	55.6	1–4
Haemonchus placei	6	66.7	1–10
Pseudostertagia bullosa	4	44.4	10–40
*Trichuris discolor***	6	66.7	1–12
Skrjabinema ovis	8	88.9	10–1026
Oesophagostomum sp.** (larva)	1	11.1	0–1

* Seven were tested for brucellosis; 5 were examined for blood parasites; and the feces of 9 were examined for coccidial oocysts. The results of these examinations were all negative.
** Tentative identification.

pronghorn antelope, according to Lucker and Dikmans (1945), has not previously been reported from bighorn sheep. As already noted, pinworms (*Skrjabinema*) have previously been reported from this host, but none from the Southwest have heretofore been definitely identified as *S. ovis*. *Oesophagostomum* has not been reported before, but the present report of its occurrence is, unfortunately, only tentative, as explained later. A provisional specific identification of whipworms from this host is given here for the first time. *Haemonchus placei* has not been reported before and the reasons for this identification of the stomach worms found also will be explained later.

Pinworms (*Skrjabinema ovis*) were the most prevalent and numerous of the parasites found (Table I). Whipworms (*Trichuris* sp., probably *T. discolor*) and large stomach worms (*Haemonchus placei*) were next in prevalence, but their numbers were small. *Cysticercus tenuicollis* was the only cestode parasite found; but it occurred in five of the nine sheep. Since carnivores are definitive hosts of this cestode, it seems probable that the numerous coyotes in the area of collection are responsible for its occurrence.

COMMENTS ON IDENTIFICATIONS

The pinworms collected in this survey agree, except for minor differences in size, with the description of *S. ovis* published by Olsen and Tolman (1950). These workers described for the first time the male of *S. parva* Dikmans, 1942 and emended the diagnosis of the genus *Skrjabinema* Wereschtschagin, 1926 as described by Mönnig (1932) to take into account their findings concerning the lips of the males of *S. ovis, S. parva,* and *S. oreamni.* These are the only species of the genus now known to be present in North America and they are all parasites of ruminants. Olsen and Tolman found that in males of *S. ovis* the free margin of each of the circumoral lips is notched medially and that this characteristic differentiates them from males of the other two species. The lips of all males examined from this collection are notched and the females also closely agree with descriptions of *S. ovis.* However, although the males collected by Allen and Kennedy (1952) also have this characteristic and agree fairly well with descriptions of *S. ovis* as to size, the tails of the few available females are much too short to be regarded as conforming to the descriptions. Therefore, since it appears that tail-length of the female may be an important specific character, Allen and Kennedy's specimens, for the present, can only be provisionally regarded as *S. ovis.*

The larva tentatively identified as *Oesophagostomum* sp. was removed from a well-developed nodule, about 7 mm. in diameter, located a short distance anterior to the ileocecal orifice. Also, in the living state, it was observed to have a well-developed buccal capsule, resembling in shape that found in *Oesophagostomum* larvae. The identification remains tentative because the specimen was lost before further details of its structure could be ascertained.

The large stomach worms collected met, except in minor particulars, the structural criteria formerly considered characteristic of the "bovine strain" of *Haemonchus contortus* and now considered by Roberts, Turner and McKevett (1954) to justify recognition of this strain as a distinct species, *Haemonchus placei.*

With regard to the characteristics and probable specific identity of the whipworms, Mr. Lucker has provided the following statements: "The males have a very slender spicule, slightly expanded, or bluntly rounded, at its distal tip. The length of the spicule varies from 2.6–3.0 mm. The ruminant species of whipworms which have this type of spicule include: *Trichuris skrjabini, T. spiricollis, T. parvispiculum, T. discolor, T. gazellae,* and *T. giraffae.* The spicule length in the first of these is specified as about 1.0 mm. According to classic descriptions, *T. discolor* has a spicule ranging in length from 1.76–2.3 mm., *T. giraffae* from 2.78–3.12 mm., and *T. gazellae* from 3.23–4.15 mm. Thus, on the basis of spicule length, the specimens in question coincide with *T. giraffae.* Baer (1950) synonymized *T. parvispiculum* with *T. discolor,* thus in effect, extending the range in spicule length for

T. discolor to from 0.85 to 2.3 mm. He mentioned *T. gazelle* but did not synonymize it with *T. discolor* because of the greater spicule length assigned to the former. He suggested that *T. discolor* may be a synonym of *T. giraffae*. Apparently, however, he was unaware of a redescription of *T. giraffae* by Baskakov (1928), in which a range in spicule length of 2.78 to 3.12 mm. was assigned to that species.

"A thorough and comprehensive study of the species that have been mentioned, and others from ruminants, is needed to substantiate either their conspecificity or distinctness; should such a study show that *T. discolor* and *T. giraffae* are indistinguishable, the latter, older name will take precedence and this will be of some importance in veterinary parasitology, since *T. discolor* has been reported from domestic ruminants on several occasions in recent years. Meanwhile, in view of Baer's action, greatly extending the range in spicule length for *T. discolor* downward, a tentative upward extension of its range to 3.0 mm. to include the New Mexico specimens seems warranted."

DISCUSSION

The area in which these mountain sheep were collected is at present open to and occupied by cattle, but not by domestic sheep. Of the parasites found in this survey, all except the pinworms have been reported as occurring in or on cattle. It seems likely, therefore, that a transfer of parasites between bighorn sheep and domestic animals is taking place within the area. Because the numbers of all parasites found, except pinworms, were small, it is probable that at the time of survey no serious damage was being done by these parasites. However, some of the species found are known to be pathogenic to domestic ruminants, and it is possible that, at times of the year when climatic conditions are favorable to the spread of the nematode parasites, these species might become sufficiently numerous in bighorn sheep to become pathogenic. As far as the writer knows, the pathogenicity of *P. bullosa* and *S. ovis*, the two species found in greatest numbers in this survey, has not been tested in any host and their life histories also have not been determined.

It is apparent that the genus *Skrjabinema*, as well as the genus *Trichuris*, is in need of further study in order that the distinction between species can be made more readily and accurately. In both cases, much reliance has been placed on size differences. This is particularly true of lengths of spicules in *Trichuris* and length of tail of females in *Skrjabinema*. A study of collections from additional localities might show much more variation in size within species than is now indicated in the literature.

SUMMARY

Each of nine mountain sheep (*Ovis canadensis mexicana*) from south central New Mexico were found to be harboring one or more of the following kinds of parasites: *Dermacentor albipictus; Otobius megnini; Cysticercus tenuicollis; Haemonchus placei; Pseudostertagia bullosa; Trichuris* sp., tentatively identified as *T. discolor; Skrjabinema ovis;* and a nodular worm larva tentatively identified as *Oesophagostomum* sp. Of these parasites, *P. bullosa, T. discolor* and nodular worm larvae have not previously been reported from this host. Pinworms identified specifically as *S. ovis* have not previously been reported from mountain sheep of the Southwest.

All nine of the sheep were examined for coccidial oocysts, by fecal examination; five were examined for blood protozoa; and seven were tested for brucellosis. The results of these examinations and tests were negative.

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SOME HELMINTH PARASITES OF FRESH AND BRACKISH WATER FISHES FROM LOUISIANA AND PANAMA*

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Most of the helminths herein reported were taken from Lake Pontchartrain and at Edgard, Louisiana during 1952–1954. The trematodes from Panama were kindly donated by Dr. R. V. Bangham.

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Lake Pontchartrain and its fauna are of interest because the water is brackish. The distribution of the species of marine and fresh-water fish which occur together is partly dependent upon wind conditions and amounts of rainfall in the drainage systems discharging into the lake. These factors tend to change the salinity of the lake and thus affect the distribution of intermediate as well as definitive hosts. In general, it was found that fresh-water fishes in the lake harbored fresh-water parasites, and the marine fishes fresh-water as well as marine parasites.

Unless otherwise indicated, all measurements are in millimeters.

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PHYLUM PLATYHELMINTHES

CLASS TREMATODA

ASPIDOGASTRIDAE Poche, 1907

Cotylogaster occidentalis Nickerson, 1902

Host: *Aplodinotus grunniens* Raf., fresh-water drum.
Location: Intestine of 3 of 5 hosts.
Locality: Lake Pontchartrain, Louisiana.

This parasite species has been reported from *A. grunniens* from the Great Lakes region by Nickerson (1902) and Dickerman (1948) and in Mississippi by Simer (1929). Dickerman (1948) reported that mature and immature *C. occidentalis* from pleurocerid snails of the genus *Goniobasis* were capable of infecting *A. grunniens*. Kelly (1927) reported *C. occidentalis* from unionid clams, *Lampsilis luteola*. Stomach analyses of hosts from Lake Pontchartrain demonstrated that *A. grunniens* was largely a clam eater, the clam ingested being always a macterid clam of the genus *Rangia*. There is a possibility, however, that *A. grunniens* acquired *C. occidentalis* not in the brackish environment of Lake Pontchartrain, but before entering the lake from fresh-water streams.

BUCEPHALIDAE Poche, 1907

Bucephaloides trichiuri n. sp.
(Fig. 1)

Diagnosis (based on two specimens, measurements on one): *Bucephaloides:* body elongate, with rounded ends; length 1.18, maximum width at level of mouth 0.30. Anterior sucker 0.12 by 0.11. Mouth 0.41 from anterior end of body. Pharynx 0.09 by 0.05; intestine saccular, pyriform, extending backward to posterior edge of ovary. Genital pore subterminal, near posterior end of body, with minute spines around its edge. Cirrus-sac about 1/6 body length. Genital atrium 0.06 long; cirrus-sac and genital atrium 0.26 long; ratio of length of genital atrium to total length of cirrus-sac and atrium, 1 : 4. Testes tandem, near midline, anterior testis immediately posterior to cecum; anterior testis 0.15 by 0.12; posterior testis 0.12 by 0.07. Vitelline follicles round, in two widely separated lateral rows, extending from level of ovary nearly to anterior sucker, 15 to 16 follicles in each row. Ovary 0.09 by 0.07; near middle of body, to right of midcecum. Uterus extending backward from ovary, looping around each testis, to right of cirrus-sac; not extending posterior to genital atrium. Eggs (collapsed) 0.017 by 0.013. Excretory vesicle extending to level of pharynx.
Host: *Trichiurus lepturus* Linn., cutlass fish.
Location: Pyloric ceca.
Locality: Lake Pontchartrain, Louisiana.
Holotype: U. S. Nat. Mus. Helm. Coll. No. 37,484.

This species is most closely related to *Bucephaloides gracilescens* (Rud., 1819) Hopkins, 1954, from which it differs in that the excretory vesicle extends to the pharynx, whereas in *B. gracilescens* it extends only to the posterior tip of the intestine; the uterus in *B. trichiuri* does not extend forward past the ovary, but in *B. gracilescens* it extends anterior to the ovary. *B. trichiuri* is named after its host, *Trichiurus*.

DIPLOSTOMATIDAE Poirier, 1886

Diplostomum sp.

Host: *Dorosoma cepedianum* (Le Seur), gizzard shad. New host record.
Location: Vitreous humor of eyes of all 35 hosts examined.
Locality: Bonnet Carre Spillway, Louisiana.

Neascus vancleavi (Agersborg, 1926) Hughes, 1928

Hosts: *Mollinesia latipinna* Le Seur, sail-fin molly; and *Lepomis macrochirus* Raf., sunfish.
Location: Brood sacs of all female *Mollinesia* examined, and around heart of 7 to 15 specimens of *L. macrochirus* examined.
Locality: Edgard, Louisiana.

CLINOSTOMATIDAE Lühe, 1901
Clinostomum sp.

Host: *Dorosoma cepedianum* (Le Seur), gizzard shad.
Location: Conjunctival sacs of 3 of 35 hosts examined.
Locality: Bonnet Carre Spillway, Louisiana.

LEPOCREADIIDAE Nicoll, 1935

Homalometron pearsei (Hunter and Bangham, 1932) Manter, 1947
Host: *Aplodinotus grunniens* Raf., fresh-water drum.
Location: Intestine of 1 of 5 hosts examined.
Locality: Lake Pontchartrain, Louisiana.

FELLODISTOMATIDAE Odhner, 1911
Fellodistomum mendezi n. sp.
(Fig. 2)

Diagnosis (based chiefly on a single favorable specimen; numerous contracted specimens were in the collection): *Fellodistomum:* body flattened, elongate, tapering at ends, 0.68 in length, 0.31 in maximum width at acetabulum. Oral sucker sub-terminal, 0.12 wide by 0.10 long. Acetabulum 0.17 by 0.17, in anterior half of body, aperture round in all specimens. Ratio of suckers 1:1.4. Prepharynx absent; pharynx oval, 0.05 by 0.06; esophagus very short or absent; ceca at first transverse, then bending posteriorly and ending at testes. Genital pore ventral, median, immediately posterior to intestinal bifurcation. Testes paired, slightly irregular in shape, lateral, post-equatorial, anterior margin of left testis slightly anterior to right testis and almost reaching acetabulum; testes twice as long as wide, left testis 0.18 by 0.09, right testis 0.17 by 0.09. Cirrus-sac median, oval, measuring 0.10 by 0.06, overlapping anterior half of acetabulum. Vitelline glands large, irregularly shaped, extending from mid-length of oral sucker to testes, not quite confluent at level of intestinal bifurcation. Vitelline ducts filled with rather transparent vitelline cells; forming a shallow V with vertex directed posteriorly between testes and ends reaching anterior border of testes. Ovary subtriangular, dextral, between right testis and acetabulum; broader than long; 0.027 by 0.074. Uterus consisting of one intertesticular loop extending to posterior end of body. Eggs 0.046 by 0.037 (average of 10). Excretory vesicle Y-shaped, bifurcating immediately posterior to ovary, with anterior branches extending to mid-acetabulum.

Host: *Brachyrhaphis episcopi* (Steindachner), top minnow.
Location: Intestine.
Locality: Gatun Lake, Panama.
Holotype: U. S. Nat. Mus. Helm. Coll. No. 37,482.

This species is named in honor of Dr. Alejandro Mendez, director of the "Museo Nacional de Panama," and Dean of Sciences at the University of Panama.

There are eight species in the genus *Fellodistomum* Stafford, 1904: *F. incisum* (Rud., 1809) Stafford, 1904; *F. agnotum* Nicoll, 1909; *F. fellis* (Olsson, 1868) Nicoll, 1909; *F. furciger* (Olsson, 1868) Yamaguti, 1953; *F. magnum* (Manter, 1934) Yamaguti, 1953; *F. profundum* (Manter, 1934) Yamaguti, 1953; *F. sebastodis* Yamaguti and Matumura, 1942; *F. preovaricum* Caballero, Bravo and Grocott, 1952.

Fellodistomum mendezi differs from the closest related species, *F. agnotum* Nicoll, 1909, in several points. The genital pore is median, but slightly to one side in *F. agnotum;* a mature specimen of *F. mendezi* measures 0.68 in length as compared with 3.30 for *F. agnotum;* vitellaria extend from the testes to mid-level of oral sucker in *F. mendezi,* from level of acetabulum to the intestinal bifurcation in *F. agnotum;* the testes in *F. mendezi* are much larger and more elongate than in *F. agnotum;* and the ceca are narrower in *F. mendezi* than in *F. agnotum. F. mendezi* resembles *F. agnotum* and *F. furciger* in that the uterus has only one coil in the posterior part of the body. It differs from all other species in the genus with respect to the distribution of the vitellaria; in no other species do the vitellaria ex-

tend from the testes to overlap the oral sucker. The species of *Fellodistomum* may be separated by the following key. *F. incisum* is not included because of its inadequate description.

Key to Species of Fellodistomum

I. Vitellaria entirely anterior to posterior margin of acetabulum.
  a. ovary anterior to acetabulum ..... *F. preovaricum*
  a'. ovary posterior to acetabulum ..... b.
  b. sucker ratio 1:2 ..... *F. fellis*
  b'. sucker ratio 1:1.5 ..... *F. agnotum*
II. Vitellaria entirely posterior to acetabulum.
  a. uterus with one coil posterior to ovary ..... *F. furciger*
  a'. uterus with more than one coil posterior to ovary ..... b.
  b. sucker ratio 1:2; body length 6 to 7 mm. ..... *F. magnum*
  b'. sucker ratio 1:1.5; body length 1.7 to 2 mm. ..... *F. profundum*
III. Vitellaria extending anterior and posterior to acetabulum.
  a. uterus with only one coil posterior to ovary ..... *F. mendezi*
  a'. numerous uterine coils posterior to ovary ..... *F. sebastodis*

GORGODERIDAE Looss, 1901
Phyllodistomum superbum Stafford, 1904

Host: *Micropogon undulatus* Linn., croaker, a brackish water fish. New host record.
Location: Urinary bladder.
Locality: Lake Pontchartrain, Louisiana.

PLAGIORCHIDAE Lühe, 1901
Macroderoides typicus (Winfield, 1929) Van Cleave and Mueller, 1932

Host: *Amia calva* Linn., fresh-water dog fish or bow-fin.
Location: Spiral valve of 4 of 6 hosts, 50 to 150 *M. typicus* per host.
Locality: Edgard, Louisiana.

CRYPTOGONOMIDAE Ciurea, 1933
Neochasmus ictaluri n. sp.
(Fig. 3)

Diagnosis (based on 17 specimens. Average measurements in parentheses): *Neochasmus:* body tongue-shaped, spined at least to posterior level of testes; length 0.52 to 0.74 (0.68); maximum width near acetabulum 0.26 to 0.32 (0.27). Oral sucker terminal, 0.04 to 0.09 (0.07) in diameter; with conspicuous crown of 22 spines 0.02 long. Acetabulum about 0.08 to 0.09 long and 0.07 to 0.08 wide. Sucker ratio 1:1.13. Prepharynx approximately one-fourth length of pharynx. Pharynx globular, 0.04 in diameter. Esophagus about three times length of prepharynx. Ends of ceca not visible. Testes round or nearly so, paired, lateral, equatorial. Seminal vesicle free, sinuous, extending from gonotyl to posterior border of acetabulum. Gonotyl ventral to anterior edge of acetabulum, with two lateral lobes (Fig. 4) having shape of two apposed boxing gloves. Seminal receptacle approximately size of pharynx, sinistral, dorsal to ovary. Ovary median, intercecal, postacetabular, pretesticular, just anterior to mid-body, with many finger-like lateral lobes. Vitellaria lateral, extending from posterior edge of testes to anterior edge of acetabulum, follicles irregular in shape. Uterus with descending and ascending limbs, filling hinder part of body almost completely. Eggs approximately 0.018 by 0.012.
Host: *Ictalurus furcatus* Le Seur, chuckle-headed catfish.
Location: Intestine.
Locality: Lake Pontchartrain, Louisiana.
Holotype: U. S. Nat. Mus. Helm. Coll. No. 37,483.

This species is named after its host *Ictalurus*.

Other species in this genus are: *N. umbellus* Van Cleave and Mueller, 1932, from *Micropterus salmoides* Lacépède; *N. microvatus* (Tubangui, 1928) Tubangui and Masiluñgan, 1944 (syn. *Metadena microvata* Tubangui, 1928; *M. ovata* Tubangui and Francisco, 1930) from *Glossogobius giurus, Glossogobius biocellatus* and *Pristipoma hasta; N. labeosus* Bennett, 1935 from a water-snake, *Natrix rhombifera.*

The possession of 22 oral spines separates *Neochasmus ictaluri* from all other

species in the genus. *N. umbellus* has 27 spines; for *N. microvatus*, Tubangui and Masiluñgan (1944) reported 7 spines in one specimen and 16 in the other; *N. labeosus* has 34 spines. The seminal receptacle of *N. ictaluri* is much smaller than that of *N. microvatus*, and in this respect, *N. ictaluri* resembles *N. umbellus* and *N. labeosus*. *N. ictaluri* differs from *N. umbellus* in its conspicuous gonotyl, and from *N. labeosus* in that mid-acetabulum lies in the first third rather than in the first fourth of the body; and the sucker ratio is 1 : 1.13 as compared with 1 : 0.71.

HEMIURIDAE Lühe, 1901

Halipegus genarchellus (Travassos, Artigas and Pereira, 1928) Manter, 1934
Host: *Roeboides guatemalensis* (Günther), sardina. New host record.
Location: Intestine.
Locality: Gatun Lake, Panama.
Specimen in U. S. N. M. Collection, 37,485.

Morphologically the Panamanian specimens resemble *H. genarchellus* as described by Travassos *et al.* (1928) except for certain small differences. The Panamanian specimens are 1.56 to 1.84 long as compared with 2.1 to 4.0 in the Brazilian specimens. In the Panamanian specimens the polar filament is 1.6 times the length of the egg (Fig. 6), while in the Brazilian specimens the polar filament is 4 times longer than the egg.

In view of the general similarity of *H. genarchellus* to *Derogenes tropicus* Manter, 1936, the type specimen of the latter, loaned by Mr. Allen McIntosh of the U. S. National Museum, was restudied in collaboration with Dr. H. W. Manter with particular reference to egg filaments. It was found that some of the eggs, at least, did possess a short, recurved filament. A filament was not visible on most of the eggs although if sharply recurved on the top or bottom surface it would be difficult to observe. Variations observed are shown in Figure 7. This species, *D. tropicus*, should be transferred to the genus *Halipegus* Looss, 1899 and becomes *H. tropicus* (Manter, 1936) n. comb. *H. tropicus* is closely related to *H. genarchellus*, *H. dubius*, and *H. parvus*, all described by Travassos, Artigas, and Pereira (1928) from Brazilian fresh-water fishes.

H. tropicus differs from *H. genarchellus* in that the ceca extend past the vitelline glands; it differs further in its shorter body, and in its shorter polar egg filament. It differs from *H. dubius* in its shorter body, in that the testes are ventral to the ceca (extracecal in *H. dubius*), and in its smaller eggs. It differs from *H. parvus* in its smaller body, smaller eggs, and in having an unequal sucker ratio.

CLASS CESTODA
PROTEOCEPHALIDAE La Rue, 1911
Proteocephalus ambloplitis (Leidy, 1887)

Host: *Amia calva* Linn., freshwater dogfish or bow-fin.
Location: Intestine of 5 of 6 hosts.
Locality: Edgard, Louisiana.

Lepomis macrochirus Raf. (sunfish) might be an intermediate host in this area as one plerocercoid was removed from the intestinal mesentery.

Proteocephalus macrocephalus (Creplin, 1825) La Rue, 1911

Host: *Anguilla rostrata* Le Seur, eel.
Location: Intestine of one of two hosts.
Locality: Edgard, Louisiana.

HAPLOBOTHRIIDAE Meggitt, 1924

Haplobothrium globuliforme Cooper, 1914

Hosts: Amia calva Linn., freshwater dogfish or bow-fin. *Anguilla rostrata* Le Seur, eel; new host record.
Location: Intestine of 4 of 6 *Amia;* intestine of 1 of 2 *Anguilla.*
Locality: Edgard, Louisiana.

PHYLUM ACANTHOCEPHALA

RHADINORHYNCHIDAE Travassos, 1923

Leptorhynchoides thecatus (Linton, 1891)

Host: Amia calva Linn., freshwater dogfish.
Location: Intestine of 1 of 6 hosts.
Locality: Edgard, Louisiana.

Telosentis tenuicornis (Linton, 1892) Van Cleave, 1947

Host: Micropogon undulatus Linn., croaker.
Location: Rectum.
Locality: Lake Pontchartrain, Louisiana.

Infections with *T. tenuicornis* were studied in *Micropogon* specimens of varying sizes. Twenty-five host specimens in each of 7 size ranges were examined, each group covering a range of 25 mm. in length. In each group, the incidence and intensity of infection were noted. Results are shown in Table I.

TABLE I.—*Incidence and intensity of infection of* Telosentis tenuicornis *in* Micropogon undulatus *grouped according to size of host*

Size of host in mm.	10–25	26–50	51–75	76–100	101–125	126–150	151–175
Number of hosts	25	25	25	25	25	25	25
Number infected	0	0	1	1	6	14	17
Average number of worms	0	0	3	7	7.3	26.2	16.5

These data show (1) that *Micropogon* did not acquire *T. tenuicornis* until it reached a size of 51 to 100 mm., and even at this point the infection was probably accidental because only 2 of 50 specimens were infected; (2) that the infection was cumulative with the highest incidence in the largest hosts; and (3) that the intensity decreased in the 151 to 175 mm. class although the incidence reached its highest peak.

PHYLUM NEMATODA

THELAZIIDAE Railliet, 1916

Haplonema immutatum Ward and Magath, 1916

Host: Amia calva Linn., freshwater dogfish.
Location: Intestine of all of 6 hosts.
Locality: Edgard, Louisiana.

CAMALLANIDAE Railliet and Henry, 1915

Spirocamallanus pereirai (Annereaux, 1946) Olsen, 1952

Host: Micropogon undulatus Linn., croaker. New host record.
Location: Posterior third of intestine.
Locality: Lake Pontchartrain, Louisiana. New locality record.

Infections with *S. pereirai* in *Micropogon* specimens of varying size ranges were studied. Twenty-five host specimens in each of 7 size ranges were examined, each group covering a range of 25 mm. in length. In each group the incidence and intensity of infection were noted. Results are shown in Table II.

TABLE II.—*Incidence and intensity of infection of* Spirocamallanus pereirai *in* Micropogon undulatus *grouped according to size of host*

Size of host in mm.	10–25	26–50	51–75	76–100	101–125	126–150	151–175
Number of hosts	25	25	25	25	25	25	25
Number infected	5	8	18	15	20	13	5
Average number of worms	1	1.75	4.9	3.4	6.6	8.3	6.6

These figures indicate (1) that *Micropogon* becomes infected with *S. pereirai* while very young, (2) that incidence of infection increases to a maximum in hosts 51 to 125 mm. long, then declines in larger specimens; and (3) the intensity of infection is light until the hosts are more than 50 mm. long, increasing markedly in hosts over 100 mm. but retaining its level even when incidence is falling.

SUMMARY

Nine species of trematodes, 3 of cestodes, and 2 of nematodes are reported from fishes in Louisiana. Two species of trematodes are reported from fishes in Gatun Lake, Panama.

New species described: *Bucephaloides trichiuri* (BUCEPHALIDAE) from *Trichiurus lepturus* in Louisiana; *Fellodistomum mendezi* (FELLODISTOMATIDAE) from *Brachyrhaphis episcopi* in Gatun Lake, Panama; *Neochasmus ictaluri* (CRYPTOGONOMIDAE) from *Ictalurus furcatus* in Lake Pontchartrain, Louisiana.

New combination: *Halipegus tropicus* (Manter, 1936), synonym, *Derogenes tropicus* Manter, 1936.

New host records: *Diplostomum sp.* (DIPLOSTOMATIDAE) from eyes of dorosomid fishes in Louisiana; *Phyllodistomum superbum* Staff., 1904 (GORGODERIDAE) from *Micropogon undulatus* in Louisiana; *Halipegus genarchellus* (TRAVASSOS, Artigas and Pereira, 1928) Manter, 1934 (HEMIURIDAE) from *Roeboides guatemalensis* in Gatun Lake, Panama; *Haplobothrium globuliforme* Cooper, 1914 (CESTODA, PSEUDOPHYLLIDEA) from *Anguilla rostrata* in Louisiana; *Spirocamallanus pereirai* (Annereaux, 1946) Olson, 1952 (NEMATODA, CAMALLANIDAE) from *Micropogon undulatus* in Louisiana.

Relationship of host size to the incidence and intensity of infection with *Spirocamallanus pereirai* (NEMATODA) and *Telosentis tenuicornis* (ACANTHOCEPHALA) in *Micropogon undulatus* was studied.

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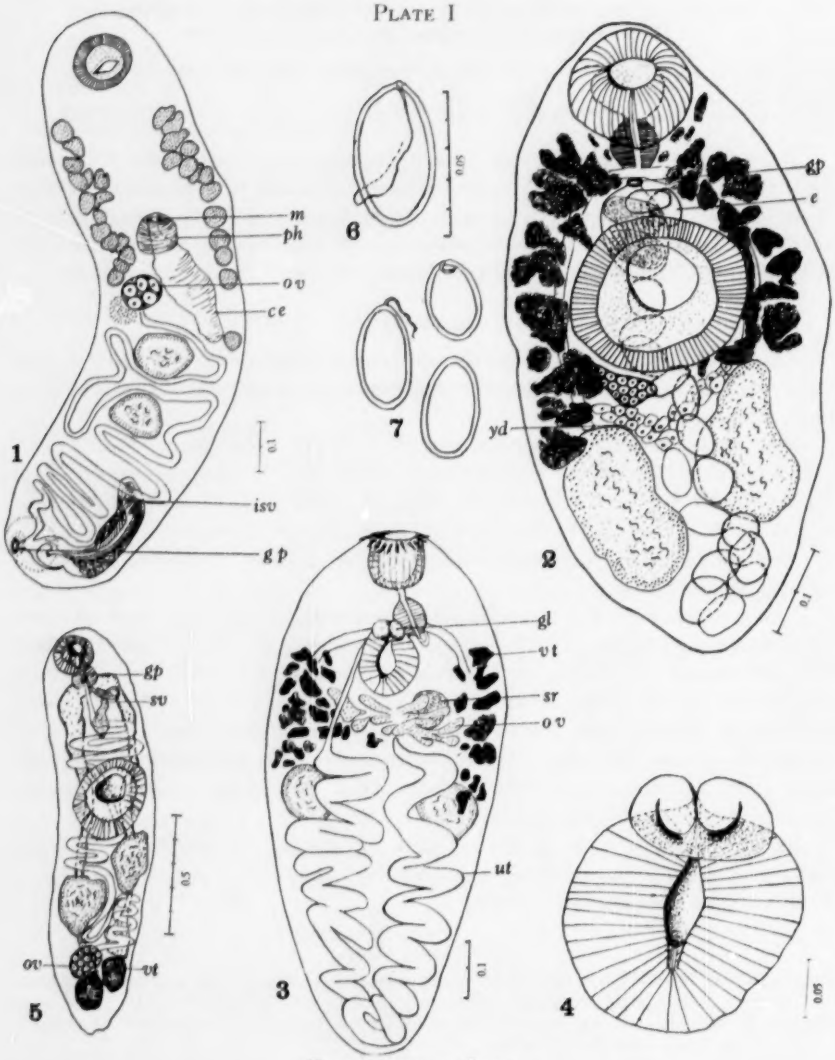
PLATE I



EXPLANATION OF PLATE

All figures were drawn with the aid of a camera lucida.

Abbreviations used: ce, cecum; e, egg; gl, gonotyl; gp, genital pore; isv, internal seminal vesicle; m, mouth; ov, ovary; sr, seminal receptacle; sv, seminal vesicle; ut, uterus; vt, vitellaria; yd, yolk duct.

FIG. 1. *Bucephaloides trichiuri* n. sp., ventral view.

FIG. 2. *Fellodistomum mendezi* n. sp., ventral view.

FIG. 3. *Neochasmus ictaluri* n. sp., ventral view.

FIG. 4. Acetabulum and gonotyl of *Neochasmus ictaluri*, ventral view.

FIG. 5. *Halipegus genarchellus* (Travassos, Artigas and Pereira, 1928) Manter, 1934; ventral view.

FIG. 6. Egg of *Halipegus genarchellus*.

FIG. 7. Eggs of *Halipegus tropicus* (Manter, 1936).

GLUCOSE CONSUMPTION OF *PLASMODIUM HEXAMERIUM*[1]

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Syracuse University

Despite a number of studies by investigators in different parts of the world, our knowledge of the physiology of the malaria parasites is still fragmentary. This is true even of the species of *Plasmodium* causing human malaria; of most of the others we know little or nothing, although some of those infecting monkeys and birds have been rather intensively studied.

Plasmodium hexamerium is still one of the less known species, but it produces good infections in ducklings and therefore lends itself to research of various kinds. It is also representative of a natural subgroup of malaria plasmodia, characterized morphologically by the production of a rather small number of merozoites per segmenter (generally 8 or less), by elongate gametocytes, and by small size. Periodicity of asexual reproduction is not marked in the group, and the infections produced lack (in the natural host species) a sharply marked crisis, and tend to be very chronic.

This subgroup has been little studied physiologically, and for this reason *Plasmodium hexamerium* was selected for an investigation of its rate of glucose consumption. Previous studies of this type dealt only with a few of the larger species of plasmodia.

MATERIALS AND METHODS

The methods employed were essentially those used in two similar studies (Manwell and Feigelson, 1949; Warren and Manwell, 1954) done in this laboratory. White Pekin ducklings, generally not more than a week old, were used as experimental animals. Infections were produced by the inoculation of infected blood into the jugular vein, the infective dose having been taken by intracardial puncture from a donor duckling, in which the parasite count was still rising (to avoid possible effects of a developing immunity).

The strain of parasite was originally isolated from a Lincoln's sparrow (*Melospiza lincolni lincolni*) caught in Fayetteville, N. Y., on May 15, 1947, and since designated as the "10D" strain by the Committee on Terminology of Avian Malaria of the American Society of Parasitologists. The main characteristics of infections produced by this strain in ducklings have been previously described (Manwell, 1952). Rather large parasite inocula, running from 100 to 400 million, were used in these experiments.

Batches of blood for glucose determinations were withdrawn by intracardial puncture, immediately removed to a constant temperature (40°C.) warm room, and placed in rocker dilution tubes of the type used in the Harvard culture process; these tubes were then treated as in this process, being agitated on a rocking table, and supplied with a constant current of a mixture of 95 percent air and 5 percent CO_2. Samples on which glucose values were to be determined were removed every 20 minutes for a period of three hours, centrifuged with 10 percent tungstic acid, and kept in the refrigerator at 4°C. until the next day, when they could be run off.

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[1] Aided in part by a grant-in-aid from the American Philosophical Society.

The Folin-Malmros method (Folin and Malmros, 1929; Klenshoj and Hubbard, 1939) was used, but with ferric duponal substituted for the gum ghatti originally specified. Duplicate determinations were made in each case.

For making red cell counts, the bright line haemocytometer was used. Reticulocytes and parasites were counted in J. S. B.-stained films, and parasitemia levels calculated in terms of the parasite number per 10,000 erythrocytes, enough parasites being always counted to keep the probable error within 10 percent.

After the completion of tests for glucose consumption, fresh ducklings were always inoculated with the remaining infective blood to make sure that the parasites were still normally viable.

Determinations of the rate of glucose consumption of normal mature erythrocytes were made from blood taken from uninfected young ducklings of similar age to those used for malaria infections. The rate of glucose consumption of reticulocytes was determined on blood from ducklings made anemic by phenylhydrazine. For this purpose each dose, amounting to 2.7 mg. of the drug per 100 gms. of body weight, was given orally on each of three successive days. Such ducks showed a reticulocytosis of almost 100 percent at the conclusion of treatment, although they showed an almost complete recovery within three or four days. These results may be compared with those of Hewitt (1937) who treated canaries with this drug. In general, the effects were very similar, except that the anemia required two or three more days to develop and recovery was also a little slower.

Since it is convenient to express the glucose consumption of malaria parasites in terms of their surface area, polar planimeter measurements were made of a number of parasites selected at random on ordinary J. S. B.-stained films. When the results were compared with values calculated on the assumption that the organisms are rectangular, it was found that there was very close agreement, and the formula "total area = 2ab" ("a" and "b" being length and breadth) was thereafter used, it being assumed that the parasites have two identical surfaces, but no thickness. The assumption of Silverman, *et al.* (1944), that parasites (*Plasmodium gallinaceum*) could be thought of as flattened disks, or of Warren and Manwell (1954) that they (*Plasmodium relictum*) could be conceived of as flattened elliptical disks, was found not to hold for *Plasmodium hexamerium*. Undoubtedly living parasites differ somewhat in dimensions from those on stained films, but some observations of the former, using a phase-contrast microscope, indicated that such differences are probably not great.

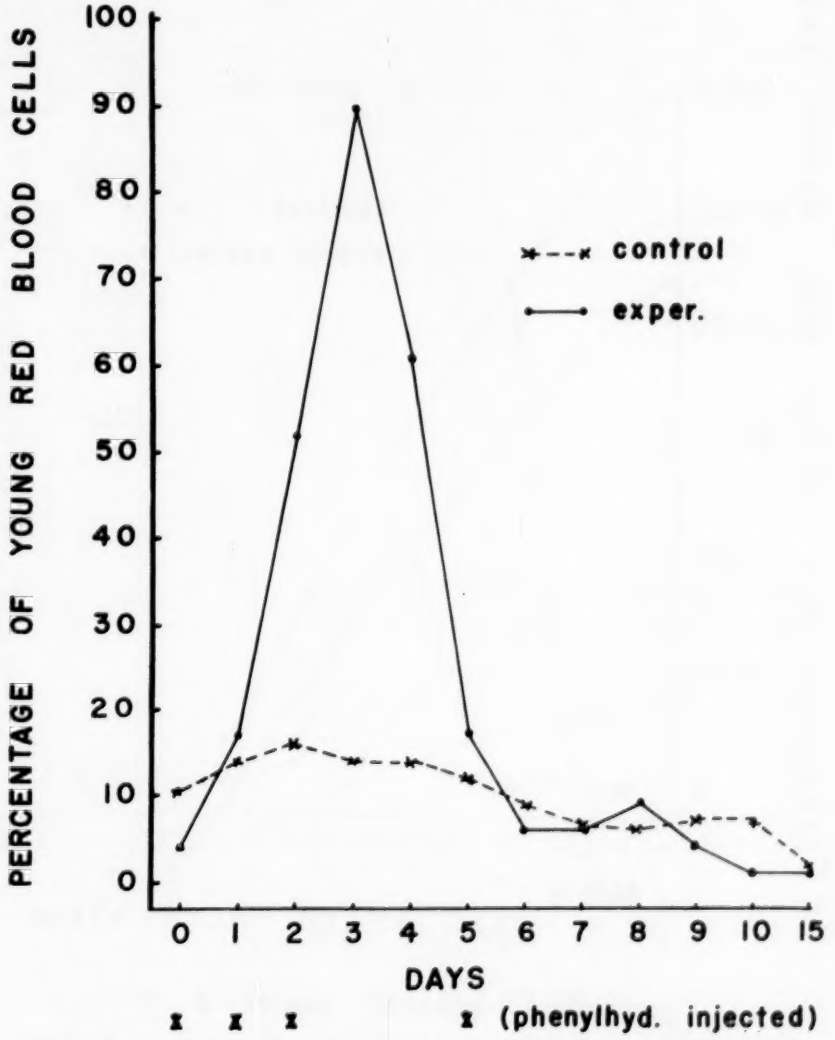
Since some observations had indicated that *Plasmodium hexamerium* may invade mature red cells in preference to reticulocytes, two series of phenylhydrazine-treated ducklings were inoculated, the one concurrently with the first injection of the drug, and the second just after the treatments were completed. The course of infection was followed in both groups, and compared with that in controls.

RESULTS

It was soon noted that the behavior of the parasite had itself been somewhat changed in the course of the rather long period in which it has been carried in ducks in our laboratory. During this time, amounting to 8 years and involving more than 400 serial passages, it has become so well adapted to life in the duckling that it regularly produces a parasitemia of 50 percent or more, and sometimes even exceeds 100 percent, due to frequent multiple invasion of the erythrocytes. This is in marked

contrast to the very low-grade, and sometimes completely inapparent infections which it produced when the first attempts were made to transfer it to this host species.

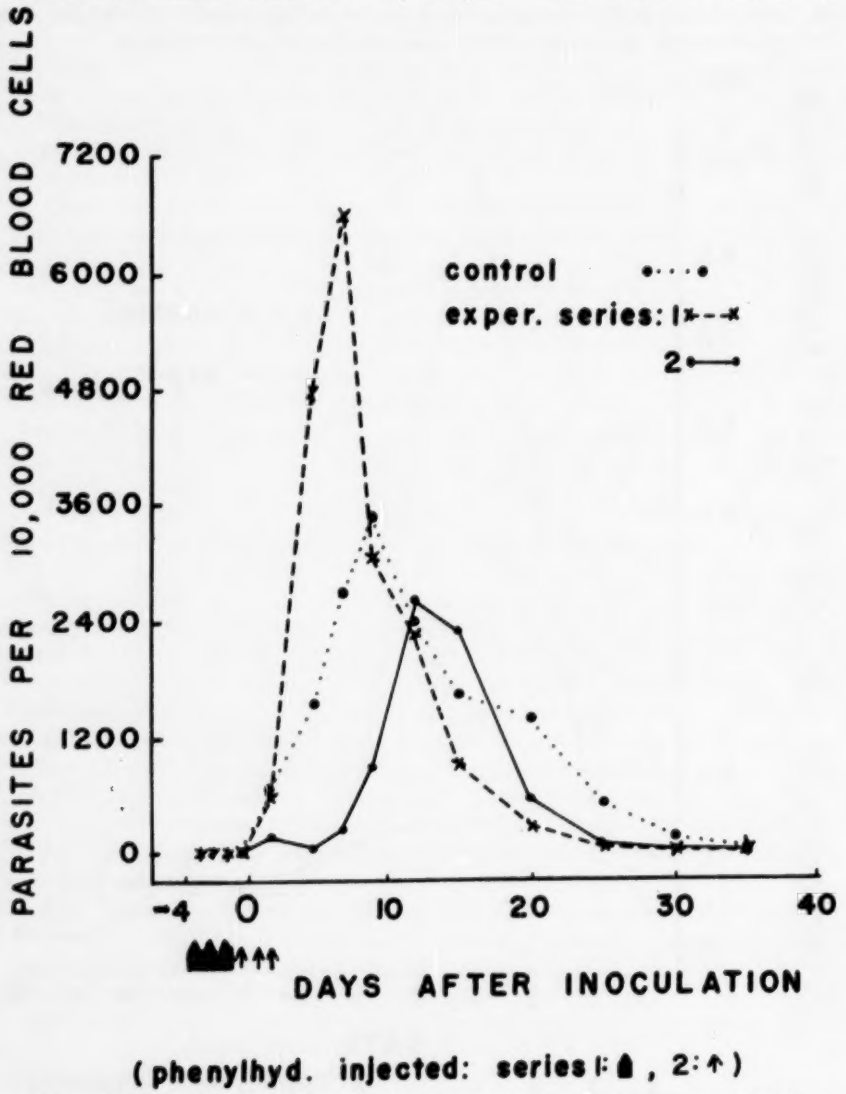
A further change, perhaps correlated with the greater intensity of infection, is an apparent increase in the average merozoite brood per segmenter. This is now 7.68 $\pm$ 0.12, instead of the usual six, from which the species name is derived.



GRAPH 1. Effect of phenylhydrazine on the peripheral blood picture of White Pekin ducklings.

The behavior of the parasite in phenylhydrazine-treated ducks (Graph 1) yielded interesting results. The infection which resulted from the inoculation of parasites concurrently with the initial dose of the drug (thus coinciding with the maximum

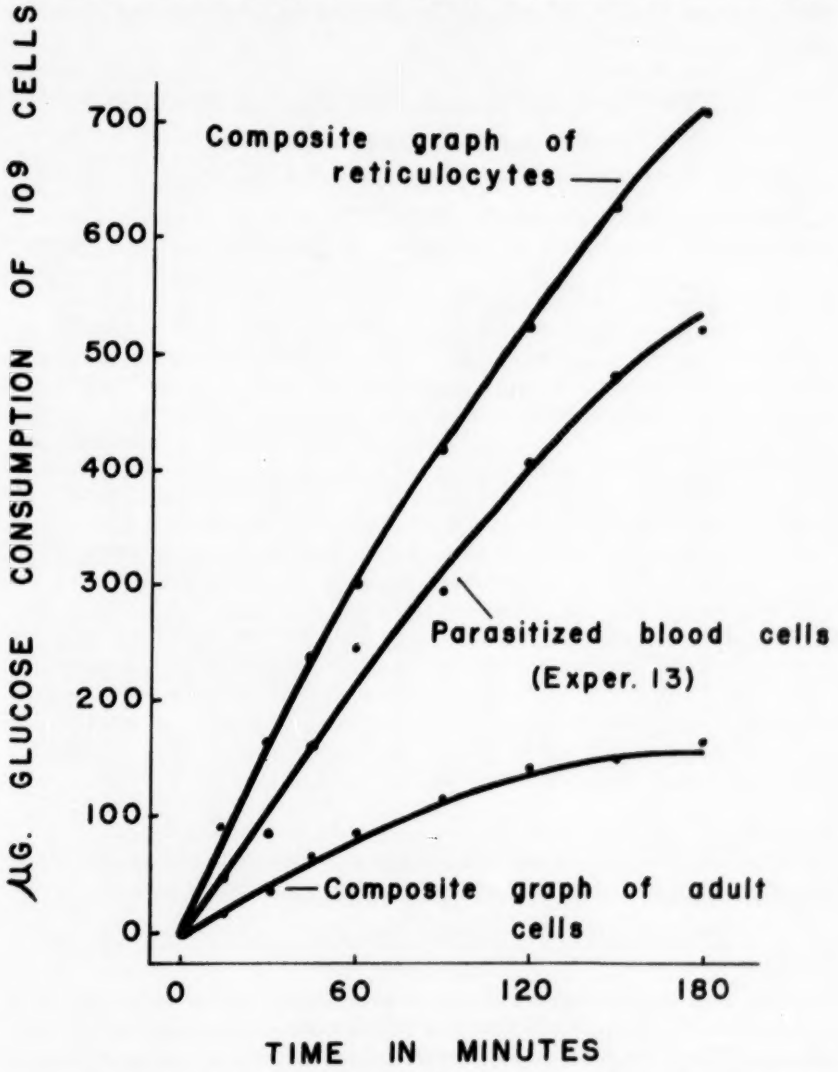
reticulocyte count) was much milder than the one which resulted from the later parasite inoculation (Graph 2), thus apparently showing the preference of this species of *Plasmodium* for the older erythrocytes. It will be recalled that the life



GRAPH 2. The behavior of *Plasmodium hexamerium* in phenylhydrazine-treated ducklings: In Series 1 the drug was given first three days prior to inoculation with parasites; in Series 2 drug administration was started concurrently with parasite inoculation.

span of the duck erythrocyte is only about 10 days (McConnell, *et al.*, 1953) and that maturation of these cells is rapid. It is possible that this peculiarity in part explains the relative non-pathogenicity of *Plasmodium hexamerium*.

Glucose determinations followed essentially the same pattern as that already found for other species of malaria parasite. Graph 3 shows curves for the consumption rates of mature erythrocytes, reticulocytes, and parasites. A small, but prob-



GRAPH 3. Composite graph showing rate of glucose consumption in reticulocytes, mature red cells, and blood parasitized by *Plasmodium hexamerium* (this curve is based on a single, but typical experiment).

ably not significant, difference in *in vivo* blood sugar levels was found as between normal, anemic, and *hexamerium*-infected ducklings (147 mg. percent, 185 mg. percent, and 170 mg. percent respectively).

Table 1 shows the summarized data of all these experiments. Mature erythrocytes were found to have a mean hourly glucose consumption rate of 75.8 ± 1.0 micrograms per hour per 10^9 cells,[2] reticulocytes had a mean rate of 366 ± 21 micrograms per hour per 10^9 cells, and for parasites the hourly rate per 10^7 square micra of area was calculated as $142 \times 10^{-3} \pm 16 \times 10^{-3}$ micrograms.

Dimensions of parasites (in square micra) were found to be, on the average: ring—4.4; trophozoite and schizont—20.4; segmenter—40.4; and gametocyte—33.8.

TABLE I.—SUMMARY OF THE EXPERIMENTAL DATA

Glucose determinations on normal and malarial (Plasmodium hexamerium) *blood in ducks*

A. Normal Blood

Expt.	E	A	R	Mg. glucose/hr./ ml. blood	Mg. glucose/hr./ 10^9 adult cells
5	2.660	0.995	0.005	2.00×10^{-1}	7.70×10^{-2}
7	2.340	0.995	0.005	2.34×10^{-1}	7.60×10^{-2}
8	2.490	0.995	0.005	1.78×10^{-1}	7.87×10^{-2}
9	2.345	0.995	0.005	1.76×10^{-1}	7.49×10^{-2}
14	2.660	0.995	0.005	1.92×10^{-1}	7.22×10^{-2}
Mean:	2.487	0.995	0.005	1.96×10^{-1}	7.58×10^{-2} S. E. $= \pm 0.10 \times 10^{-2}$

B. Anemic Blood

Expt.	E	A	R	Mg. glucose/hr./ ml. blood	Mg. glucose/hr./ 10^9 adult cells
20	1.242	0.056	0.944	4.80×10^{-1}	4.05×10^{-1}
21	1.017	0.068	0.932	3.60×10^{-1}	3.98×10^{-1}
22	1.010	0.046	0.954	3.10×10^{-1}	3.18×10^{-1}
23	1.000	0.044	0.956	3.00×10^{-1}	3.11×10^{-1}
24	1.012	0.044	0.956	3.20×10^{-1}	3.98×10^{-1}
Mean:	1.056	0.052	0.948	3.54×10^{-1}	3.66×10^{-1} S. E. $= \pm 0.21 \times 10^{-1}$

C. Parasitized Blood*

Expt.	E	A	R	P	a	b	c	d	Mg. glucose/hr./ ml. blood	Mg. glucose/hr./ 10^7 μ^2 parasite area
2	2.120	0.995	0.005	23.7	37	54	4	6	2.70×10^{-1}	1.33×10^{-4}
3	2.138	0.900	0.100	45.5	55	38	3	4	5.18×10^{-1}	2.38×10^{-4}
4	2.080	0.990	0.010	19.5	50	42	3	5	2.10×10^{-1}	0.95×10^{-4}
6	1.992	0.864	0.136	24.5	55	38	3	4	2.90×10^{-1}	0.98×10^{-4}
10	2.088	0.864	0.136	25.2	61	32	4	3	3.20×10^{-1}	1.31×10^{-4}
11	1.861	0.880	0.120	38.5	54	41	2	3	3.26×10^{-1}	1.33×10^{-4}
12	1.520	0.888	0.112	38.1	66	28	0	6	2.60×10^{-1}	1.55×10^{-4}
13	1.495	0.922	0.078	31.0	50	46	0	4	2.40×10^{-1}	1.55×10^{-4}
Mean:	1.911	0.913	0.087	30.8	54	40	2	4	3.04×10^{-1}	1.42×10^{-4} S. E. $= \pm 0.16 \times 10^{-4}$

Key to symbols:

$E = \frac{\text{erythrocytes per mm}^3}{10^6}$ $\quad R = \frac{\text{reticulocytes}}{\text{total red cells}}$ $\quad A = \frac{\text{adult erythrocytes}}{\text{total red cells}}$

P = percent parasitemia

a = % type "a" parasites
b = % type "b" parasites
c = % type "c" parasites
d = % type "d" parasites

* In all experiments, an attempt was made to secure blood for test in which the infection was close to its peak, in order to have a high parasite count. Since individual infections vary somewhat, the time at which the blood samples were taken necessarily also varied.

Although these figures represent the mean values for the parasite in the duck, it is well to remember that parasite sizes in a different host would probably differ, and might very likely be less, just as in passerine birds (probably the most frequent natural hosts of *Plasmodium hexamerium*) the average number of merozoites per segmenter seems to be usually smaller.

The rates of glucose consumption already given show the average mature red cell to have a metabolic rate about one-fifth that of a reticulocyte, and an "average

[2] For purposes of this investigation, it was assumed that the leucocytes and thrombocytes consume relatively negligible quantities of glucose.

parasite" (one having a surface area of 24.8 square micra) consumes about the same quantity as a reticulocyte in unit time, or 35.5×10^{-11} mg. per hour.

DISCUSSION

The apparent demonstration that *Plasmodium hexamerium* prefers older erythrocytes as host cells (perhaps it would be more accurate to say that such cells may be more hospitable to the parasite, or that they may be easier for it to invade) is of considerable interest, since most of the species of malaria plasmodia so far studied are more commonly seen in the younger red cells. Using phenylhydrazine-treated birds, Hewitt (1939) found that *Plasmodium cathemerium*, for example, produced much heavier infections because of the over abundance of reticulocytes. Conceivably, the drug might exert a direct effect on the parasites themselves (or even on the defense mechanism of the host) but neither Hewitt nor others who have used the drug believe this to be the case.

It is logical to ask whether it is proper to speak of the consumption of glucose by malarial parasites at all, since they are within the erythrocytes, and whatever reaches them must come from the interior of the host cell. Perhaps the red cell (especially when it is nucleated, as in birds) may at least initiate the chemical transformations of the well-known Krebs' cycle. No final answer can be given to this question, and even the study of the metabolic behavior of parasites freed from their host cells (Fulton, 1939) does not give conclusive results. He found that such parasites (*Plasmodium knowlesi*) could utilize a variety of sugars, and also glycerol, but it is still possible to question whether they are not behaving differently than they would in their normal environment of cell stroma and hemoglobin.

Nevertheless, since parasites freed from their host cells have been shown to be able to utilize glucose and some other sugars directly, and since *Plasmodium hexamerium*-infected red cells consume glucose much faster than uninfected mature erythrocytes of apparently similar age, it seems very probable that the plasmodia themselves are consuming glucose as such. Furthermore, Speck and Evans (1945) were able to show that cell-free preparations of *Plasmodium gallinaceum* contained enzymes essential to the phosphorylation of glucose, and McKee (1951) in an extensive review of the carbohydrate metabolism of the malaria parasites, seems to concur.

The question may also be asked as to how significant data such as those herein reported really are, in view of the fact that the various techniques employed, including the basic one of glucose determination, all involve a certain degree of inherent error (Warren and Manwell, 1954). But the percentage of error at any one point in the total process is small, and as likely to occur on one side of the line as on the other. Thus such errors tend to cancel themselves out, and their importance is much minimized by basing the determinations on repeated experiments, as in this work. Nevertheless, since other techniques for the determination of glucose consumption by red cells are now available (Bartlett, *et al.*, 1955), it would undoubtedly be worth while to employ them also in future work, so that results thus obtained could be compared with those had from the more usual methods.

Among the more interesting uses of information of this kind are the comparisons it makes possible of the physiology of different species of *Plasmodium*. From the data now available it would appear that these three species rank as follows in glucose consumption rates:

Species	Mg. glucose per hour per 10^7 square micra of surface area	Authority
Plasmodium relictum	4.39×10^{-4}	Warren & Manwell, 1954
Plasmodium gallinaceum[3]	3.00×10^{-4}	Manwell & Feigelson, 1949
Plasmodium hexamerium	1.42×10^{-4}	

Although relatively large, these differences are of course small in quantitative terms, and may therefore not be significant. Because of the inherent error necessarily involved in the techniques used, it does not seem possible to put such differences to statistical test. But one may be reasonably sure that species of *Plasmodium* do differ physiologically, when they differ in so many other ways.

Since formulae were worked out for the prediction of glucose consumption by the species of plasmodia previously tested in this laboratory (see papers just cited), a corresponding formula[4] was also constructed for *Plasmodium hexamerium:*

$$W_t = 7.58E \times 10^{-2} \ (A + 4.828R + 7.493P \times 10^{-5} \ (a + 5b + 10c + 8.5d))$$

in which the symbols are those given as in a note to Table 1, and the formula derivation essentially the same as used in the previous publications already mentioned. The deviation between the mean calculated from the experimental results and the mean calculated by using the formula was found to be statistically not significant according to Student's "t" test, as P was greater than 70 percent. Such formulae may be useful in calculating the amounts of glucose likely to be required by parasites in culture.

SUMMARY

The rates of glucose consumption have been determined for mature erythrocytes and reticulocytes of the duckling, and for duckling erythrocytes parasitized by *Plasmodium hexamerium*. Mean values per hour were found to be respectively: 7.58×10^{-2} mg. per 10^9, 3.66×10^{-1} mg. per 10^9, and 1.42×10^{-4} mg. per 10^7 square micra of parasite area.

The mean areas, in square micra, of parasites of different ages and types were found to be: ring, 4.4; trophoxoite and schizont, 20.4; segmenter, 40.4; gametocyte, 33.8. The "average parasite" (having an area of 24.8 square micra) consumed 3.53×10^{-11} mg. glucose per hour, or about five times as much as a normal adult red cell. Evidence was obtained that this species of malaria parasite has a preference for mature or older red cells, although it also invades reticulocytes.

Though differences are absolutely small, and may not be significant, experimental data indicate that *Plasmodium hexamerium* has a lower glucose consumption rate than do *Plasmodium relictum* and *P. gallinaceum*.

[3] Values obtained for this species by Silverman *et al.* (1944), were lower, but their methods differed in several important respects.

[4] The symbols used are those given at the bottom of Table 1, except for W_t, which is the total glucose consumption (mg.) per hour per ml. of parasitized blood.

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STUDIES ON LEISHMANIASIS IN THE GOLDEN HAMSTER[1]

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Certain of the animal agents of disease offer unique possibilities for the study of general problems of host response to infection. Moreover, one might expect that such a system, particularly in the case of the intracellular parasites, would help to elucidate some of the unsolved problems of the physiology of the intact potential host animal. Even such gross behavioral characteristics as life-cycles of parasites with varied physiological needs at definite time intervals imply qualitative and/or quantitative nutritional relationships between host and parasite. This is perhaps most clearly illustrated in the case of malaria where the parasite, at different stages of development, utilizes tissue cells of dissimilar embryonic origin and of different physical, chemical and metabolic constitution.

The present investigation is a preliminary survey of some host-parasite relationships of an intracellular infectious agent, *Leishmania donovani*,[3] and the Golden hamster. In the latter, the parasite produces an intense continuous stress of fatal outcome.

MATERIALS AND METHODS

The work reported in this paper was carried out on Golden hamsters (60 day old males) which were obtained from a commercial breeder and maintained on a diet of Purina dog chow pellets supplemented with greens.

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[1] Portion of a thesis submitted as partial fulfillment of the requirements for the degree of Doctor of Philosophy, University of California at Los Angeles. This work was done under the direction of Dr. Gordon H. Ball, Department of Zoology, whose kindly advice and assistance are gratefully ackowledged by the author. This investigation was carried out (in part) while the author held a U. S. Public Health Fellowship (pre-doctoral, 1950–1951).

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[3] Kindly supplied by Dr. L. A. Stauber. Department of Zoology, Rutgers University.

Preparation of the inoculum: The donor hamster (having been infected 50–60 days previously, was sacrificed, its spleen aseptically removed and weighed in a sterile tared vessel. Impression smears were taken and stained with Giemsa in order to confirm the infection. All donor spleens were heavily parasitized having more than 20 parasites per spleen cell nucleus. The spleen was then placed in a calibrated, sterile Ten Broeck tissue grinder and slowly comminuted in sterile physiological saline. Dosages were calculated so that each experimental host would receive an equivalent of 20 mg. of donor spleen per 100 gm. of body weight. Although the actual number of parasites probably varied considerably among the experiments, all of the hamsters, in a particular experiment, received inocula from the same donor spleen. Also, the results indicated that, within the limits of the work reported here, the actual or absolute number of parasites did not significantly alter the course of the infection. It was found that the procedure reported here regularly resulted in the death of the animal in 60 to 70 days. As a result of this observation, animals were sacrificed on days: 1, 5, 10, 20, 35 and 55. In one experiment, an equivalent dosage of non-parasitized homologous spleen was prepared by pooling a number of these organs. All inoculations were administered by the intraperitoneal route.

Organ weights, with the exception of the adrenals, were taken on a pharmaceutical type torsion balance. Adrenals were weighed on a Roller-Smith torsion balance (250 mg. capacity).

Livers were dried to a constant weight (at 100° C), pulverized and analyzed for total nitrogen by the Pregl micro-Kjeldahl method. All samples were run in duplicate.

The following tissues were fixed in Bouin's fixative and stained with Delafield's hematoxylin: spleen, kidney, adrenal and liver.

RESULTS

Organ weights: Spleens of non-infected animals showed little variation in weights over the experimental period; the ratio of spleen-weight to body-weight remained throughout at about 1 milligram of spleen-weight per gram of body-weight. Liver-weights of uninfected hamsters exhibited a decline relative to the body-weight as the animals grew. Kidney-weights paralleled body-weight increases keeping the ratio of kidney-weight to body-weight fairly constant. Normal adrenal-weights also tended to parallel body-weights, but there was somewhat greater variability in weight relationships of these organs than was found among other organs studied (Table I).

Both absolute and relative weight increases were noted in all organs taken from animals infected with *L. donovani* (Table II). Most striking of these changes was that of the spleen which, over a period of 55 days, increased in weight by a factor of more than 8. This increase could be observed as early as 10 days after inoculation and served as a good index of the progress of the disease. Infected livers exhibited hypertrophy which was evident at 10 days of infection, but was less striking than that of the spleen.

Increase of kidney weight was observed late in the infection as a presage of fatal outcome. The kidneys do not harbor the parasite.

Perhaps the most significant organ hypertrophy, from the standpoint of pathogenesis, was that of the adrenals which, like the kidneys, do not ordinarily harbor

TABLE I.—*Organ weights and ratios of organ weight to body weight of normal uninfected hamsters**

	Day 1**	Day 5	Day 10	Day 20	Day 35	Day 55
Body weight (gm)	88.7	97.3	92.3	104.7	124.7	123.7
Liver weight (gm)	4.06	4.30	4.08	4.07	5.28	4.85
Liver ratio***	45.9	44.0	44.3	38.9	41.5	39.2
Spleen weight (mg)	91.0	122.5	78.9	119.9	126.5	120.6
Spleen ratio***	1.02	1.28	0.82	1.04	1.04	0.97
Kidney weight (mg)	763	741	775	792	989	873
Kidney ratio***	8.62	7.60	8.40	7.57	7.84	7.07
Adrenal weight (mg)	12.9	13.4	14.4	14.5	19.2	21.6
Adrenal ratio****	14.8	14.0	15.7	13.8	15.2	17.4

* Each figure represents the mean value of 5 animals.
** Animals were 60 days of age on day 0.
*** Expressed as milligrams per gram of body weight.
**** Expressed as milligrams per 100 grams of body weight.

L. donovani. Although there was some variability in the adrenal weights and ratios of uninfected animals, the results indicated a significant increase of infected hamster adrenals which was evident after 35 days of infection (Table II). In

TABLE II.—*Organ weights and ratios of organ weight to body weight of hamsters infected with* Leishmania donovani*

	Day 1**	Day 5	Day 10	Day 20	Day 35	Day 55
Body weight (gm.)	88.3	97.8	100.9	107.8	119.6	115.3
Liver weight (gm.)	3.82	4.30	5.02	5.06	5.56	6.54
Liver ratio***	43.3	44.5	49.4	47.1	46.4	56.6
Spleen weight (mg.)	107.5	121.4	227.9	342.3	672.4	1006.8
Spleen ratio***	1.24	1.26	2.23	3.15	5.65	8.75
Kidney weight (mg.)	778	820	821	895	896	1122
Kidney ratio***	8.79	8.48	8.04	8.24	7.68	9.82
Adrenal weight (mg.)	14.3	13.8	14.3	17.9	24.6	39.7
Adrenal ratio****	16.4	14.1	14.1	16.7	20.5	34.6

* Each figure represents the mean value of 5 animals injected with 20 mg. of infected spleen per 100 gm. of host (I. P.).
** Animals were 60 days of age on day 0 (day of infection).
*** Expressed as milligrams per gram of body weight.
**** Expressed as milligrams per 100 grams of body weight.

order to determine the possible effect of the inoculum itself, the above experiment was repeated with an additional control of equivalent dosage of pooled spleen emulsion from uninfected donor hamsters. It seemed that the inoculation of normal hamster spleen had a slight enhancing effect on the adrenal weights, but this did

TABLE III.—*Effect of inoculation of normal hamster spleen emulsion on adrenal weights and ratios of adrenal weight to body weight compared with uninoculated and infected groups**

	Day 5	Day 10	Day 20	Day 35	Day 55
	*INJECTED GROUP***				
Body weight (gm.)	77.7	82.6	93.4	102.2	106.7
Adrenal weight (mg.)	17.8	19.1	19.5	22.3	27.1
Adrenal ratio***	22.7	23.1	21.2	21.9	25.4
	*UNINOCULATED GROUP***				
Body weight (gm.)	77.9	83.2	95.8	104.4	107.9
Adrenal weight (mg.)	15.6	17.2	18.7	22.6	28.2
Adrenal ratio***	20.2	20.6	19.7	21.7	26.2
	*INFECTED GROUP***				
Body weight (gm.)	77.9	83.2	92.5	102.4	105.3
Adrenal weight (mg.)	19.5	19.9	22.9	31.7	41.3
Adrenal ratio***	25.1	24.0	24.9	30.9	39.5

* Each figure represents the mean value of 4 animals.
** Animals were 60 days of age on day 0. The inoculated groups received 20 mg. per 100 gm. of body weight of uninfected homologous spleen or infected donor spleen.
*** Expressed as milligrams per 100 grams of body weight.

not persist beyond 10 days. It was also apparent that the marked hypertrophy mentioned above was related to the disease process rather than to the inoculation of homologous tissue (Table III and Figure 1).

Liver nitrogen: It was found that the water content of infected livers varied from about 1 to 3% higher than the livers of uninfected hamsters.

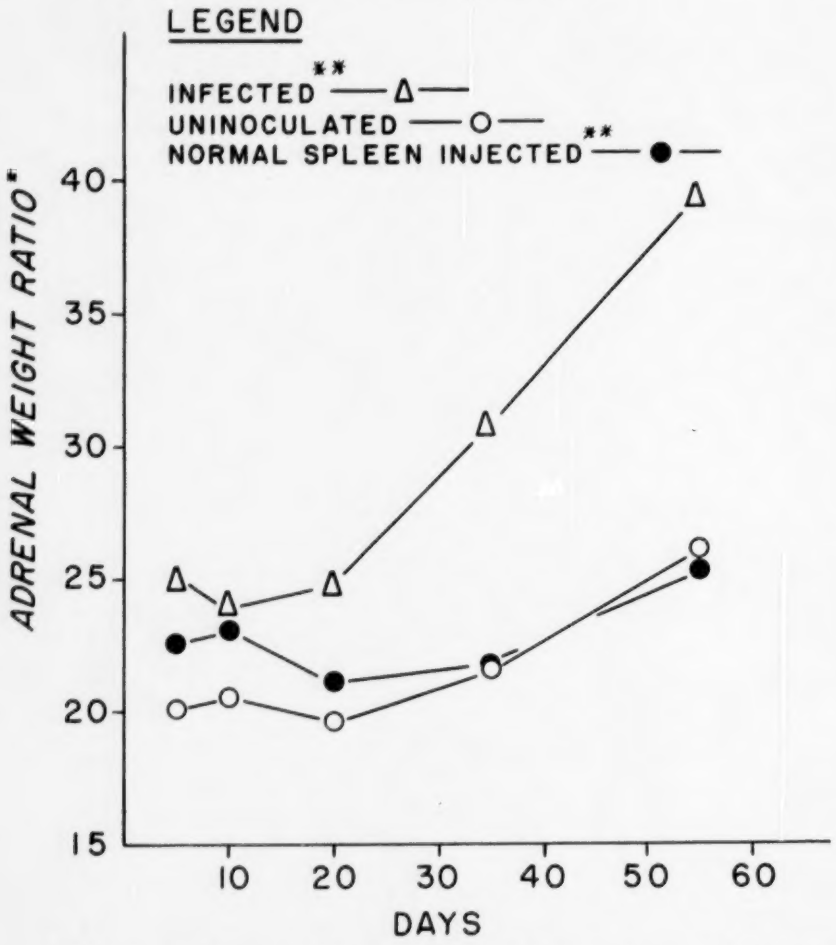

FIG. 1.—Adrenal weight-ratios of hamsters infected with *L. donovani* compared with adrenal weight-ratios of uninfected and homologous spleen injected animals.

* Expressed as milligrams of spleen per 100 grams of body weight. Each point represents the average of 4 animals.

** Dosage: 20 mg. of donor spleen per 100 gm. of body weight.

It is evident from Table IV that, with the exception of day 5, the average nitrogen content (dry weight) of infected livers was slightly higher than that of uninfected livers. The maximum increment was seen on day 55 of infection and was

about 2.4% greater. Since the hypertrophy of infected livers steadily increased up to 34.8% at 55 days, there was a gross increase in liver nitrogen. It appears, however, that there was no significant increase in the absolute liver nitrogen content (dry weight) of hamsters infected with *L. donovani*. The slightly higher values of liver nitrogen and water content in infected hamsters might have been due to alteration of blood volume of this organ by the disease process itself. The blood volume of normal liver has been estimated at about 3% of the total weight.

Pathology: The tissue pathology of Kala-azar in the Golden hamster was essentially similar to that described by Meleney (1925) for the Chinese hamster and, for that reason, will not be discussed in detail in the present report. The Golden hamster rapidly developed disease which always proved fatal while the Chinese hamster tolerated the infection remarkably well over a long period of time.

A large number of the infected hamsters used in the present study, if allowed to survive beyond the usual 55 day term of the experiment, developed a rapidly progressive, terminal edema as first described by Goodwin (1945). The edema was so massive as to cause an increase in body weight of about 100% in some cases. The bulk of the fluid was to be found in the fascia and in the body cavity (Plate 1). Fluid did not extend to the limbs or to the head.

TABLE IV.—*Liver nitrogen of hamsters infected with* Leishmania donovani *compared with nitrogen content of uninfected hamster livers**

	*INFECTED GROUP***					
	Day 1	Day 5	Day 10	Day 20	Day 35	Day 55
Liver weight (gm.)***	3.82	4.30	5.02	5.06	5.56	6.54
Total nitrogen (%)****	10.67	9.58	9.57	10.36	10.69	11.09
	*UNINFECTED GROUP***					
	Day 1	Day 5	Day 10	Day 20	Day 35	Day 55
Liver weight (gm.)***	4.06	4.30	4.08	4.08	5.29	4.85
Total nitrogen (%)****	10.15	9.68	9.40	10.09	9.51	10.73

* Each figure represents the mean value of 5 animals. All samples were done in duplicate.
** Each animal received a dose of 20 mg. of infected spleen per 100 gm. of body weight (I. P.).
*** Net weight.
**** Dry weight basis. Livers were dried to a constant weight at 100° C.

As previously indicated, kidney hypertrophy became evident by the 55th day of infection at which time there was extensive degeneration of tubules and glomeruli. Degenerative changes were first clearly observed at 35 days. Kidney damage undoubtedly played a role in the development of edema. Concomitant with the appearance of lesions in the kidney, protein was found in the urine.

The most significant pathological change was that observed in the adrenal cortex. The adrenals of hamsters infected with *L. donovani* showed signs of degeneration as early as the 20th day of infection (Plate 2, Fig. 2). These early changes were only suggestive and consisted of vacuolization of a few cells and an apparent increase in cells of the stroma. At 35 days, many vacuolated cells were seen in the reticularis and fasciculata. Some cells were undergoing cloudy degeneration (Plate 2, Fig. 3). Sections made at 55 days showed very extensive cellular degeneration in the fasciculata and reticularis (Plate 2, Fig. 4). Many areas were devoid of normal parenchymal cells. Occasional groups of intact cortical cells were seen scattered throughout the section. A few of the latter contained parasites. The glomerulosa was not noticeably affected other than a slight thinning of this zone. Degenerating cells were not seen in the glomerulosa in significant numbers.

The adrenal medulla appeared to be unaffected during the course of the disease, but sections taken at 55 days of infection showed some compression of this structure.

Gellhorn *et al.* (1946) observed amyloid in the adrenals of hamsters infected with *L. donovani* and estimated that this substance replaced over half of the cortical area. Fulton and Niven (1951) saw similar deposits of amyloid in the adrenals of cotton rats. Ritterson (1948) noted the extensive destruction of cortical cells in hamsters sacrificed late in the infection. More recently, Leathem and Stauber (1952) observed the same process, also in the Golden hamster. The changes described above do not appear to have occurred in the Chinese hamsters studied by Meleney.

DISCUSSION

Leishmania donovani produces an intense continuous stress of fatal outcome in the Golden hamster. Sayers (1950) discussed this type of stress and pointed out that, under such circumstances, adrenal glands undergo hypertrophy which is proportional to the time lapse between onset and death. The results set forth in the present report clearly indicate that this was the case in hamsters infected with *L. donovani* (Tables II & III; Figure 1). In addition, Sayers indicated that this type of stress results in a depression of sudanophilic substance and cholesterol of the adrenal cortex. This, to be sure, is the usual course of events, but the "stressed" hamster has been found to exhibit a reversal of the latter situation. Alpert (1950), using histochemical methods, found normal hamster adrenals to be poor in lipid and free of demonstrable cholesterol. Leathem and Stauber (1952), also using histochemical methods, confirmed this observation. Further, they were able to show that the adrenals of hamsters infected with *L. donovani* exhibited a "mild but definite cholesterol reaction" in the cortex four weeks after inoculation. At 42 days, the fasciculata and reticularis exhibited a strong cholesterol response which was further enhanced at 53–56 days of infection. It is apparent that the rise in cholesterol is an index of prolonged stress in the hamster. This view is substantiated by the increase in adrenal weight which has been demonstrated (Figure 1) and which is generally considered to be a valid index of stress phenomena (Sayers, 1950). Even though other mammalian adrenals respond to stress by a depression of cholesterol, the above data do not necessarily imply a fundamental difference in the pathway of synthesis of adrenal cortical hormone in hamsters. In general, it appears that organ hypertrophy is a more reliable index of adrenal response to prolonged stress than are chemical changes.

There is good evidence that chronic Kala-azar in man may result in adrenal cortical damage similar to that observed in the hamster. This possibility was suggested by Bramachari (1942) on the basis of the development of skin pigmentation in this disease. Chakravarty *et al.* (1949b) showed that one-third of their well developed cases of Kala-azar excreted abnormally high amounts of urinary chlorides concomitant with a decrease in plasma chloride. Corkill (1948) indicated that the stress of battle experience activated sub-clinical infections of Kala-azar in troops. The author (Ritterson and Stauber, 1949) has indicated that the nutritional stress of a low protein diet (10% casein) results in massive infections of individual host reticulo-endothelial cells and a decreased survival time of growing hamsters. Corkill (1949) similarly suggests that this is true in human Kala-azar.

Meleney (1925) has shown that *L. donovani* infection results in extensive destruction of liver parenchymal tissue in the Chinese hamster which is also true in the Golden hamster. However, since liver damage is not accompanied by any decrease in absolute nitrogen content (dry weight) (Table IV) one would expect that replacement of parenchymal cells, largely by parasitized macrophages, tends to cancel-out any possible depression of nitrogen due to destruction of liver cells. It is also true that since the bulk of the liver is increased as much as 34.8% at 55 days of infection (Table I and II), this organ exhibits a corresponding increase in gross nitrogen content as compared with normal livers. The nitrogen increase might be slightly modified by the water content of infected livers which appears to vary from 1 to 3% higher than uninfected livers. Chakravarty *et al.* (1949a), using a variety of tests, demonstrated hepatic dysfunction which was directly proportional to the severity of the disease in man. Stauber *et al.* (1954) have shown, by electrophoretic methods, that serum albumin is markedly decreased in hamsters infected with *L. donovani*. Although there were some unusual differences in other serum components of this host, the depression of albumin was consistent with that found in human infections. Hypoalbuminemia reflects the damage of liver parenchymal tissue.

Host response to infection in Kala-azar is indeed a complex phenomenon. This intracellular parasite not only effects tissue changes directly, as in the case of the liver and spleen, which is to a degree mechanical in nature, but also brings about indirect secondary changes as adrenal and kidney hypertrophy. The individual variability seen in the terminal response to this infection (e.g., whether or not edema develops) probably reflects the weakest constitutional link of the host in the chain of pathological events. When edema develops, its very grossness suggests that several influences may affect it, such as kidney damage and a depression of osmotically active serum albumin via liver damage. If the latter situation reached a critical state before adrenal "exhaustion" occurred, one would expect that the dehydration effect of this "pathological adrenalectomy" would be masked. The possibility that "stress exhaustion" of the adrenal cortex may be complicated by toxic effects should not be ignored, although the evidence for toxic effects of *L. donovani* is not yet conclusive. On the contrary, early development of the "stress induced" adrenal cortical insufficiency, which increases host susceptibility to secondary invaders, could lead to death without prior edematous changes. The host-parasite system, Golden hamster and *Leishmania donovani*, should be especially valuable as a tool for further analyses of stress phenomena in infectious disease as well as providing an additional experimental approach to the study of adrenal physiology.

From the evidence presented and cited in this paper, the author would tentatively suggest the following gross sequence of events contributory to the development of Kala-azar as a disease process: 1. Invasion and proliferation of cells of the reticulo-endothelial system. 2. Adrenal cortical hyperactivity with an increase in protein catabolism. 3. Progressive damage to liver parenchyma. 4. Progressive kidney failure and/or adrenal cortical "exhaustion."

SUMMARY

The following studies were carried out on young male hamsters: Effects of infection with *Leishmania donovani* on: 1) weights of liver, spleen, kidneys and

adrenals; 2) Liver nitrogen content and 3) Tissue changes related to pathogenesis, especially of the adrenals.

The findings were as follows: 1) weights of liver, spleen, kidneys and adrenals increased during the 55 day term of infection with *L. donovani*. 2) The liver nitrogen content of infected hamsters showed little or no increase on an absolute (dry weight) basis. 3) Massive edema was noted as a frequent terminal phenomenon. The edema was associated with liver damage and kidney failure. 4) Progressive degeneration was observed in the adrenal cortex of infected hamsters.

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Description of Plates

Plate 1.—Necropsy of hamster aftre 65 days of infection with *Leishmania donovani*. Note massive fascial edema and ascitic fluid. Splenomegaly is grossly evident.

Plate 2. Adrenals of hamsters infected with *Leishmania donovani*. (All photomicrographs were taken at 100×).

Fig. 1. Normal adrenal cortex of Golden hamster.

Fig. 2. Hamster adrenal cortex after 20 days of infection with *L. donovani*. Note vacuolization.

Fig. 3. Hamster adrenal cortex after 35 days of infection. Note cloudy degeneration and extensive vacuolization.

Fig. 4. Hamster adrenal cortex after 55 days of infection. Note absence of distinct cell boundaries. Nuclei are in various stages of degeneration.

PLATE I

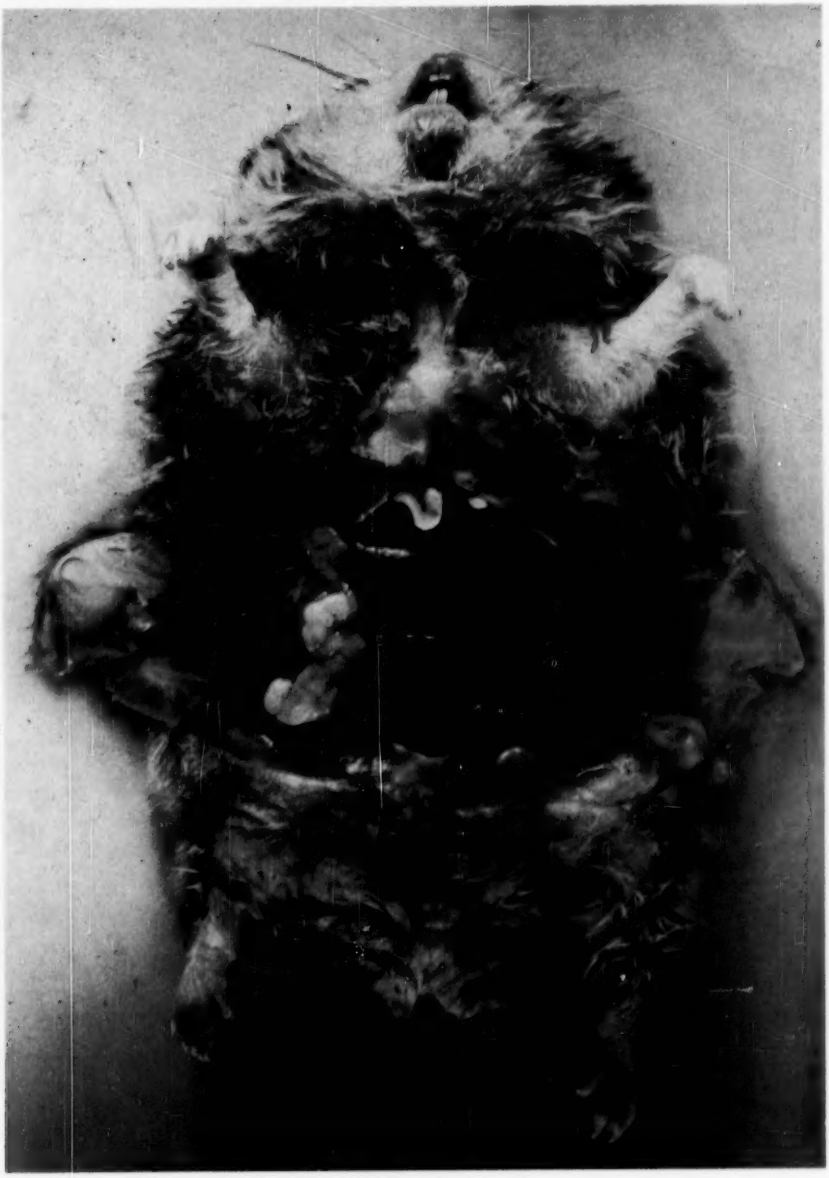
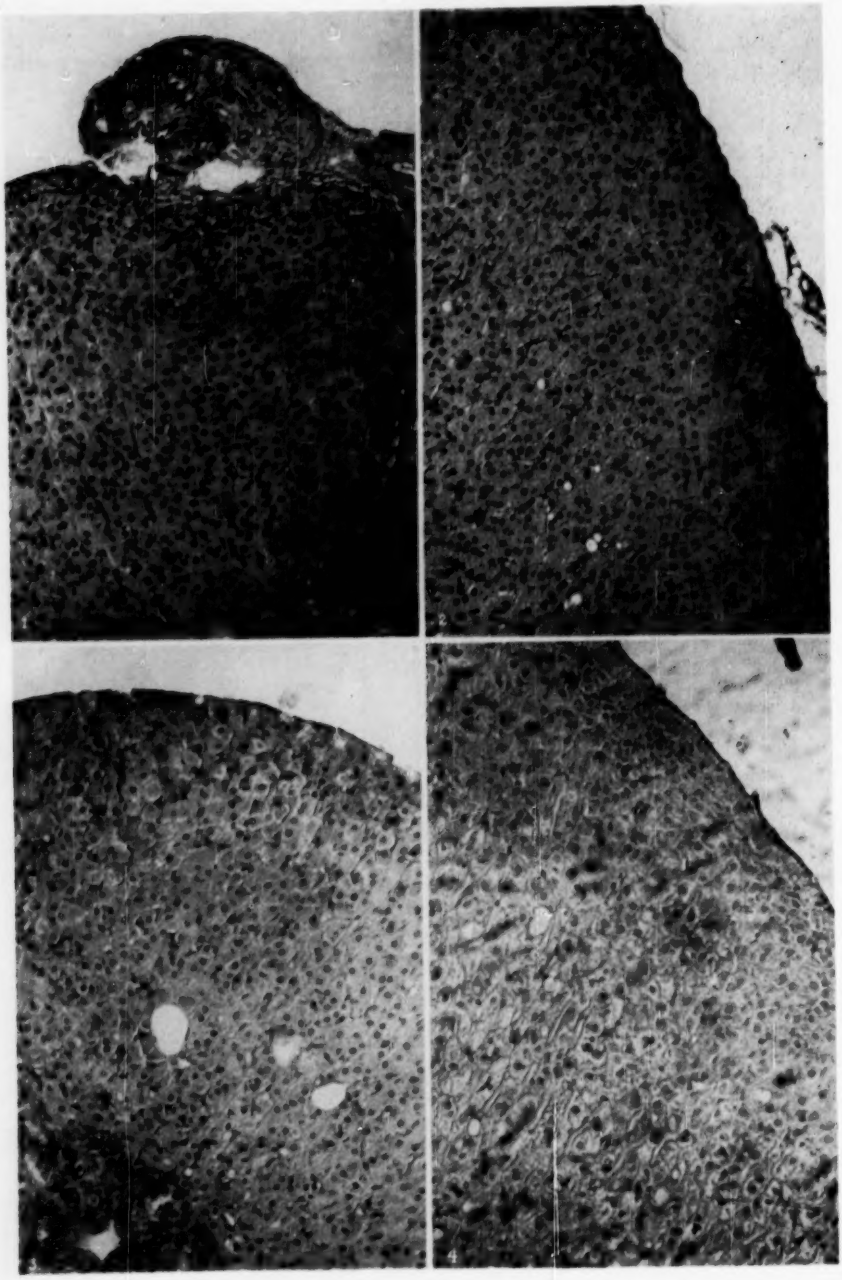


PLATE II



THE OXYGEN CONSUMPTION OF *RHABDITIS STRONGYLOIDES* AND OTHER NEMATODES RELATED TO OXYGEN TENSION

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Animals exhibit various relationships to oxygen conditions in their environments. Each physiological process proceeds within certain, sometimes narrow, limits of oxygen tension and usually exhibits some maximum of efficiency at a particular optimum oxygen tension or range. These oxygen requirements and optima are reflected in the bionomics of an organism, determining in part, the range of conditions under which it can survive and its degree of success in reproducing and maintaining populations in particular environments. The purpose of this series of experiments was to determine the effect of oxygen tension upon oxygen consumption in three small nematodes of rather divergent habit and to correlate these data with their survival.

MATERIALS AND METHODS

FORMS USED

It was proposed to use forms representing (a) a completely free-living existence (b) a free-living form which was capable of adapting itself to a parasitic environment under the proper circumstances and (c) a parasitic form. As a free-living nematode *Rhabditis elegans* was selected. This was tentatively identified by Dr. L. J. Thomas and before the identity could be confirmed, the entire culture was lost in a fire. This form is found in the soil and was obtained by soil analysis and cultured.

As a representative of a free-living form which is capable of adapting itself to a parasitic life, *Rhabditis strongyloides,* kindly identified by Dr. B. G. Chitwood, was selected. The worms used in this work are all descendants of a strain isolated and cultured by the author from pustules in the skin of a cow and reported by Levine *et al.* (1950). There are occasional reports in the literature of the invasion of the skin of dogs and rarely other animals by this form (Dikmans, 1948). Chandler, Alicata, and Chitwood (1942) consider that this adaptation represents one of the possible routes by which nematodes that migrate through the body originally became parasites of vertebrates. On the basis of this speculation we are utilizing this form as an intermediate between the parasitic and the free-living. Because of the fire which destroyed the *Rhabditis elegans* cultures, *Rhabditella axei,* a similar form though somewhat smaller, was substituted in the survival experiments.

The parasitic forms selected were larvae of the small strongyles of the horse, primarily of the genera *Cylicocyclus, Cylicocercus, Cylicostephanus* and *Cyathostomum,* recovered from horse feces. The third stage was used as the basic comparison form. These larvae are in themselves free-living (though non-feeding), but the entire cycle requires a host animal.

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After the initial isolation of the *Rhabditis* from their natural habitat, they were put on nutrient agar. Since they feed on the accompanying bacteria no effort was made to keep the cultures sterile. In order to maintain uniformity for the experiments the worms were subcultured onto blood agar plates of the following composition: Difco blood agar base with 5 percent whole horse blood added to the cooling plates. Three or four days after subculturing, the worms were ready for use. The worms may be used from the plate for several days before too many young stages appear.

The worms are collected for use in two ways. Under optimum conditions of moisture the worms climb to the lid of the Petri dish and collect in the water of condensation and may be simply washed off with saline. In other cases where it is necessary to recover them from the blood agar plates a small amount of the liquefied agar is gently scraped from the surface with a scalpel blade and placed on the screen of a "micro-Baerman" apparatus. This latter is made by putting a short length of rubber tubing and small screw clamp on the bottom of a five centimeter funnel. A piece of fine mesh silk with a wire ring woven into it is placed in the funnel. It is used by filling it to the screen with warm water and then placing the material on the screen. The worms, attracted by the warmth migrate through the screen and collect in the bottom of the tube. After 15–30 minutes a few drops are released by the clamp into a depression slide and the worms are then removed one by one with a micro-pipette. The worms are washed by transferring them one by one through several changes of saline to rid them of extraneous materials. A 0.2 percent saline was found to be best suited to the worms by observing their behavior at different concentrations, and this was used throughout the investigation.

The small strongyles were obtained by culturing manure from horses shown to be infected with these parasites.

Methods of Analysis

In considering any study of oxygen consumption *per se,* several points must be kept in mind. Rarely are other respiring forms such as bacteria eliminated completely from experimental material. This factor, while undoubtedly affecting the absolute result, can be minimized if a consistant, careful technique is followed with each experiment. The relative effect is therefore rather constant.

Among many forms it has been found that simple crowding of the animals is sufficient cause for alteration of basic physiological processes. Further, a group of animals respiring in a closed vessel could appreciably lower the oxygen tension over a relatively short period of time. In these experiments the volume of the respiratory chamber is such that with the size of the worms used, the worm-fluid volume ratio is approximately 1 : 6,000,000 per worm. It was felt that this would make negligible any lowering of oxygen tension by the worms or by crowding effect. Average wet weight was used as a basis of comparison. Since the worms were in a fluid medium it was felt that a direct analysis would be desirable. The work herein reported was done by the Winkler method. The method was chosen because it represents a simple reproducible technique with a high degree of accuracy (Allee and Oesting, 1934) and is admirably suited to the determination of dissolved oxygen.

The technique of Barth (1942) was followed, modifying it when necessary, to

suit the conditions of the experiment. This is a micro-Winkler method designed for use with fluid quantities in the order of 250 cubic mm. The method is as follows:

TB syringes, 0.25 cc, are marked with a diamond pencil on the barrel to show a 300 cubic mm mark. A small circle of lens tissue is introduced into the syringe barrel to act as a screen over the tip. The tip of the syringe is then stoppered with a cap made by closing one end of a small piece of rubber tubing. This is done with the plunger removed. The worms are removed from the saline with a micropipette and placed in the syringe from the top. The syringe is then filled with saline to the top and the plunger inserted so that no air is trapped. After removing the cap the plunger is carefully pushed in to expel fluid to the 300 cubic mm level. The syringes are recapped in such a way that no air is introduced and then set aside for the animals to respire for a given length of time. As stated previously, because of the high worm-fluid ratio it was felt that over the periods of time the worms were respiring they did not use sufficient oxygen to reduce the total available oxygen appreciably and hence affect their respiration rate. This was further borne out in that the time periods and numbers of worms varied enough to indicate that time or numbers had no apparent effect upon the rate of oxygen consumption. At the conclusion of the respiration period the chemical determination for oxygen is begun. The determinations are run in pairs with a blank control. (The detailed method of preparation of the reagents may be found in the Alsterberg modification of the Winkler given in *Standard Methods for the Examination of Water and Sewage*, 9th Ed. 1946; a summary appears below.) A second syringe is rinsed with a manganous sulfate solution allowing the tip of the syringe to retain the small amount which it holds when the plunger is in the barrel (about 5 cubic mm). The respiration syringe is then coupled to the reaction syringe with a tight coupling made from a collar of pressure tubing and the saline transferred (and mixed with the manganous sulfate) to the reaction syringe. The saline is brought to the 235 cubic mm mark (230 cubic mm saline plus 5 cubic mm manganous sulfate) and the tip wiped dry. Next, 10 cubic mm of alkaline sodium iodide with sodium azide added to prevent nitrite interference is drawn into the syringe. The resulting precipitate is evenly distributed in the syringe by twirling it between the fingers, and it is stoppered air-free and allowed to stand 4 minutes. At the end of this time it is unstoppered, the tip wiped, and 5 cubic mm of concentrated sulfuric acid drawn into the syringe to dissolve the precipitate. This frees the iodine which is subsquently titrated after transferring the fluid to a spotting plate for the titration. Titration is carried out by means of a micro-burette designed by the author (to be described elsewhere) using 1/300 N sodium thiosulfate (made fresh each time by the appropriate dilution of a standardized 0.1 N solution). A small drop of 1 percent starch solution is used as an indicator, and clearing of the solution is taken as the end point. The total volume of thiosulfate required to titrate the iodine in the saline is then recorded.

The amount of oxygen originally present is calculated in terms of the thiosulfate used. The amount of thiosulfate needed to titrate the fluid from the experimental chambers is subtracted from that needed to titrate the fluid from the control, the result being equivalent to the oxygen consumed by the worms. 1 cubic mm of thiosulfate in the burette $\times 0.00333$ (normality of the thiosulfate) $\times 5.6$ cubic mm oxygen (oxygen equivalent of thiosulfate) equals 0.0186 cubic mm oxygen. Since

the worms respire in a volume of 300 cubic mm of saline but only 230 cubic mm is transferred to the reaction syringe, it is necessary to multiply the result obtained from the product of the above factors by 30/23. This result is then the total amount of oxygen used by the worms. Dividing by the number of hours and then by the weight of the worms gives the oxygen used per unit weight per hour.

For purposes of expressing the oxygen consumption in terms of units of wet weight it is necessary to arrive at an average weight per worm. This is done by the indirect method of computing first, approximate volume, then specific gravity. To compute the volume of the worms a number of them were accurately measured from oral opening to anus and across the widest part of the body. From these measurements an average was computed. The worm was considered to be a cylinder and the volume arrived at. Several copper sulfate solutions of different specific gravities were mixed and the specific gravity of the worms noted. The mean dimensions of *Rhabditis elegans* and *R. strongyloides* are as follows: (For third stage larvae as noted previously)

Length: mouth to anus	803.6 microns
Width: across widest part of body	48.7 microns

The specific gravity of *R. elegans* and *R. strongyloides* was 1.02. Applying the formula $W = r^2 l(1.02)$ we get an average weight of 1.52 micrograms. These figures compare favorably with those of Overgaard-Nielson (1949) who employed the same method though he rounded off the specific gravity and simply considered it as 1.0. The mean dimensions of the horse strongyle larvae are as follows:

Length: anterior end to anus	544.2 microns
Width: widest part of body	43.4 microns

The specific gravity of the horse strongyle larva is approximately 1.01. The average weight is thus 0.81 microgram.

Reliability of Results

Blank samples of saline were run on two separate solutions of saline. The results of five determinations on each sample showed an experimental difference of 0.0558 cubic mm oxygen in the first series or an experimental error of 4.6 percent. The second series showed a difference of 0.0465 cubic mm oxygen or a mean experimental error of 3.5 percent. Both of these figures fall within the usually accepted standard for experimental error and reproducibility of results.

Precautions were taken to insure as accurate results as possible. The experiments were done in a building with thermostatically controlled heat so that there was not too great a temperature variation. The larvae were counted in the syringe using a binocular dissecting microscope. Every effort was made to insure accurate counts by rechecking.

Survival Studies

To determine the relation of oxygen tension to survival, the following method was used. Twenty active worms were placed in a syringe containing fluid in which the oxygen tension had been adjusted as described later. The syringe was stoppered and laid aside. It was gently agitated and the worms examined for activity at 15 minute intervals until it was observed that half of the worms no longer moved.

A determination of a 50 percent mobility makes equal allowance for weak forms tending to expire easily and the occasional resistant form which outlasts the others. The oxygen tension of the original fluid was determined by the foregoing method and calculated in the same manner. Survival could then be related to initial oxygen tension.

The procedure involved in altering the oxygen tension was the same whether it was desired to raise it or lower it, and utilized either oxygen or nitrogen depending upon the result desired. The worms, after being rinsed, were placed in a 15 cc centrifuge tube and about 5–8 cc of 0.02 percent saline added. The top was plugged with a two hole stopper fitted with two pieces of glass tubing, one of which just penetrates the stopper and is fitted on the outside with a short length of rubber tubing with a screw clamp on it. The other glass tube goes down into the centrifuge tube, extending below the surface of the saline. The outer end of this glass tube connects to the gas tank by rubber tubing and the flow of gas which bubbles through the saline is regulated by a valve on the gas cylinder. It was found that after the initial four to six minutes of gas bubbling, the speed with which the gas passed through the saline was more closely correlated with the tension than was the length of time over which the gas bubbled.

RESULTS

Rhabditis strongyloides

A total of 1103 worms were placed in the respiration chambers in 22 experiments. The number of worms in each chamber varied from 20 to 140. They were allowed to respire for periods varying from 2.5 to 7 hours. The majority of the determinations were made for a period of approximately 4 hours, the extremes in time being employed as a check against the possibility of time's having any effect upon consumption rate. Further, parallel experiments were run at the same oxygen tension with n and approximately 2n number of worms to eliminate the possibility of number's affecting the consumption rate. The worms were all actively moving when the experiment terminated. The analysis of the saline was made immediately following the termination of the respiration period. The difference in initial oxygen tension and that at the termination of the experiment due to the consumption of the worms is only a few tenths of one percent. Hence, it cannot be well argued that the worms were subjected to any great stress due to a lessening of oxygen tension. Starting with an oxygen tension of 27.9 mm Hg, there was a steady increase in oxygen consumption by the worms from 0.269 cc oxygen/gm/hr to 2.125 cc oxygen/gm/hr at an oxygen tension of 58.5 mm Hg. From this point the oxygen consumption seemed to be at a more nearly constant rate, rising to approximately 2.6 cc oxygen/gm/hr at an oxygen tension of 370 mm Hg. This is a rise of only 0.6 cc oxygen/gm/hr over a tension range of 315 mm Hg. We may thus consider the area represented by an oxygen tension of approximately 56 mm Hg as the critical tension for this particular worm. These data are shown graphically in Figure 1 with the critical tension shown by the sharp break in the curve. The critical tension is the oxygen tension below which the animal is incapable of regulating its uptake and its consumption then declines reflecting the quantity of oxygen available to it.

A curve was fitted mathematically to the data collected and two straight lines

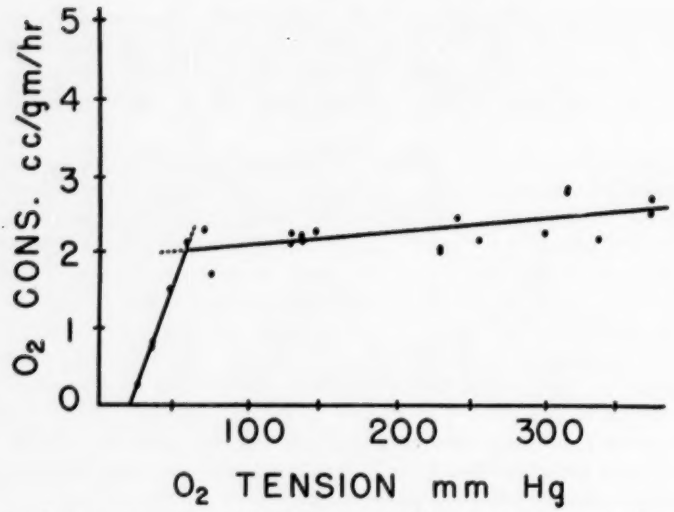

FIG. 1. Oxygen consumption of *Rhabditis strongyloides* related to oxygen tension.

appeared to give the best fit. This seems justified biologically also. The lines were derived from the equation:

$$y = mx + b$$

Rhabditis elegans

In the case of *Rhabditis elegans* a total of 1068 worms were placed in the respiration chambers in 21 separate experiments. Each experiment involved from 23 to 120 worms. The worms were allowed to respire for periods of time ranging from 1.5 to 3.5 hours. The saline was analyzed immediately following the respiration

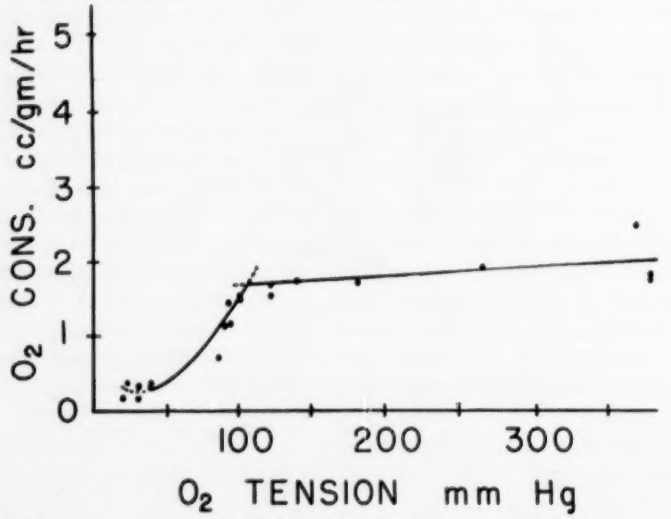

FIG. 2. Oxygen consumption of *Rhabditis elegans* related to oxygen tension.

period. In this form there was a low oxygen consumption in the order of 0.2–0.3 cc oxygen/gm/hr in the range of oxygen tensions represented by 20.7 mm Hg to 39.6 mm Hg. As the tension of oxygen was increased from this point, there was a steady increase in oxygen consumption of the worms until a tension of approximately 121.5 mm Hg was reached. At this point the consumption was 1.63 cc oxygen/gm/hr. Further increases in oxygen tension had no significant effect upon oxygen consumption of the worms. A maximum oxygen tension of 383.1 mm Hg was reached. These data are shown graphically in Figure 2, the break in the curve representing the critical tension.

The data on *Rhabditis elegans* were fitted to a curve and again it was found that the best fit involved two curves. The curves were a straight line and a parabolic section and were derived from the equations:

$$1)\ y = a + bx + cx^2$$
$$2)\ y = mx + b$$

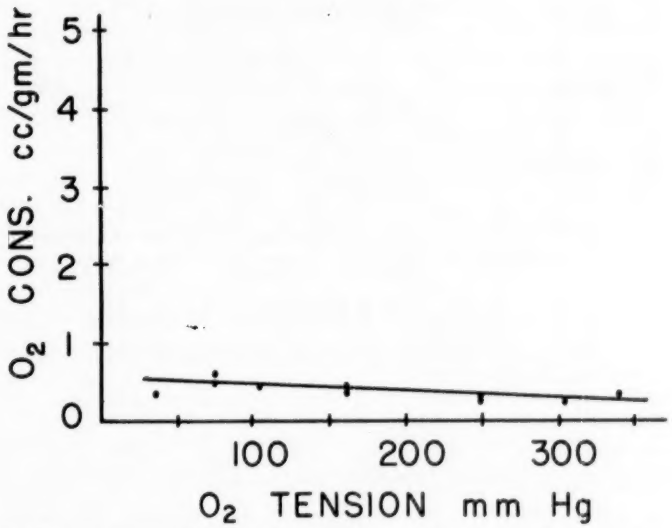

FIG. 3. Oxygen consumption of small horse strongyle larvae related to oxygen tension.

Small horse-strongyle larvae

The experiments with the small strongyle larvae involved a total of 652 worms and consisted of 12 separate determinations. The individual determinations included 20 to 130 worms each. The respiration times varied from 2.5 to 7 hours. It was found in one instance that the larvae were quite active even after 24 hours in the chamber. At all oxygen tensions tried, ranging from 37.6 mm Hg to 339.3 mm Hg, the oxygen consumption of the worms was at a minimal level, that is 0.308 to 0.469 cc oxygen/gm/hr. The data are shown graphically in Figure 3. The data on small strongyle larvae are well suited to a single straight line curve which was calculated from the equation:

$$y = mx + b$$

Survival times

Observations were made on two species of worms, *Rhabditis strongyloides* and *Rhabditella axei*, with regard to survival at varying oxygen tensions. The *Rhabditis* were observed at 6 oxygen tensions ranging from 43.8 to 343.4 mm Hg. The *Rhabditella* were observed at 5 different tensions ranging from 39.1 to 340.7 mm Hg. The 50 percent survival time was noted in both cases. Approximately 20 worms were used for each experiment, though in several preliminary runs this number was varied to eliminate the possibility that number may affect survival time. The data for the two series of experiments are shown graphically in Figure 4. Both worms

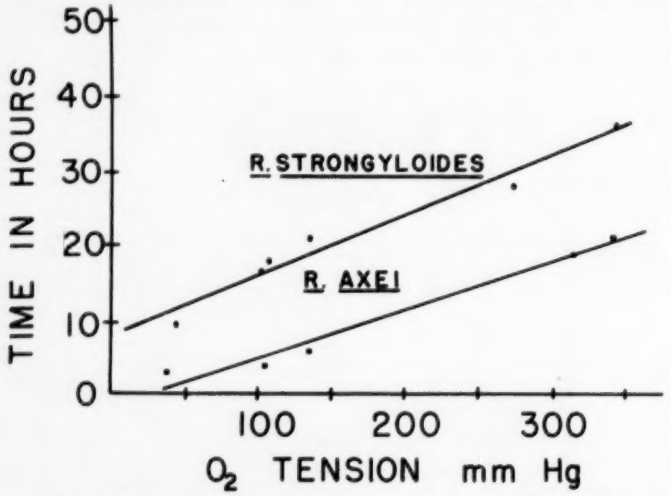

FIG. 4. Survival times of *Rhabditis strongyloides* and *Rhabditella axei* related to oxygen tension.

showed the same relation to oxygen tensions though at different levels. As the tension increased so did the survival time, reflecting the increase in oxygen available to the worms. These data were fitted to straight line curves using the following equation:

$$y = mx + b$$

DISCUSSION

Evidence indicates that intestinal worms are neither completely anaerobic nor do they live in an anaerobic environment as formerly thought (Rogers 1949a, 1949b). Von Brand and Simpson (1945) have shown that tissue forms likewise will not survive in the absence of oxygen.

These experiments have shown that there are not only definite, predictable respiration patterns among certain nematodes but that these patterns vary with the species. *Rhabditis strongyloides*, a nematode capable of either a free-living or parasitic existence, has an oxygen uptake curve (as related to oxygen tension) which indicates a very low critical tension, in the order of 58 mm Hg. *Khabditis elegans*, a completely free-living form, shows a similar curve but with a higher critical tension, approximately 130 mm Hg. The last form used for comparison, the

third stage larva of the small horse strongyle, showed complete independence of oxygen tension and an extremely low level of consumption.

The curves obtained for these nematodes indicates their ability to regulate their oxygen uptake and to a certain point, at least, maintain constant oxygen uptake regardless of external conditions. This same mechanism is found in many invertebrate animals. Harnisch (1932, 1933) in his experiments with *Fasciola hepatica* and *Ascaris lumbricoides* found that at the rather low oxygen tensions with which he worked, the oxygen consumption was dependent upon the oxygen available. The highest concentration of oxygen used however, was the amount contained in one atmosphere of air. This tension represents the lower part of the curve for *Rhabditis*. Von Brand (1947), working with larval *Eustrongylides*, subjected the worms to oxygen tensions that ran the full scale from 0.0 to 760 mm Hg. In the experiments done at approximately room temperature, he found constant oxygen consumption for the range of oxygen tensions running from 36 to 760 mm Hg, with a dropping off only at tensions lower than 36 Hg, though he shows no curves to indicate exactly the critical tension. The work herein reported tends to confirm the presence of a similar kind of regulation in other nematodes. Rogers (1949 a & b) using species of *Nematodirus* and *Haemonchus* from the sheep plotted curves for oxygen consumption in these forms at various oxygen tensions. The greatest tension at which he worked was 150 mm Hg. His curves indicate a general increase in consumption which is directly proportional to the oxygen available until a critical tension is reached, then a leveling off of the oxygen consumption takes place. The critical tensions shown for *Haemonchus* and *Nematodirus* are approximately 75 and 50 mm Hg respectively. These compare favorably with the critical tension found for *Rhabditis strongyloides*, the form adapted to a parasitic environment.

McCoy (1930) using the larvae of *Ancylostoma caninum* found that at approximately room temperature they consumed only a very small amount of oxygen, in the order of 0.0008 cubic mm/worm/hr, regardless of the oxygen tension. This observation coincides well with the work reported here on the small strongyles of the horse. The two forms are closely allied morphologically and taxonomically.

Von Brand (1946) adding his own observations to those of others has concluded that many helminths have both an aerobic and an anaerobic metabolism. He further concluded that both types of respiration can be used effectively by the same species of worm under varying conditions. Finally he believes that there are several general patterns of respiratory activity in helminths and that they are associated more with habitat than with taxonomic groupings. In other words, adaptation on the part of the worms is taking place.

It is always interesting to speculate upon the adaptive significance of physiological data relating to any given species of animal. The present study offers an opportunity for this. It has been observed in the past that many animals live rather close to their physiological limits. In the case of *Rhabditis elegans* we have an animal seemingly well adapted to life in an atmosphere with an oxygen content approximately equal to that of air. When the oxygen tension falls below this point however, we find a rapid diminution of oxygen consumption.

For *Rhabditis strongyloides*, however, a different picture is presented. In this form the critical tension is lower; this can readily be associated with the ability

of this animal to survive in the parasitic habitat. We may further speculate that the presence of a critical tension, even though lowered, may prevent this form from extending its parasitic habitat beyond the skin lesions or similar locations with an oxygen tension somewhere between that of venous and arterial blood (60–100 mm Hg). Here then is a form found in both a free-living and, to a limited extent, parasitic environment, displaying in its respiratory physiology an adaptation which seems to suit it ideally to this very existence.

In general the survival curves tend to show the more favorable environment found in the free-living existence. As the oxygen available to the worms increases, so does their survival. If any argument for distribution can be made from these data, it is in the direction of an ever increasing movement toward the free-living environment and not the parasitic. It is known however, that an animal's distribution is governed not by a single favorable factor but rather by the net result of several factors.

Among the small strongyle larvae still a different sort of adaptation is seen. No critical tension is evident and consumption is at a uniformly low level. The biology of this worm shows at once the need for such an adaptation. The worm must be able to survive for long periods of time in the air with a minimum of moisture so that an active metabolism would needlessly use stored food reserves. Upon being ingested by the host it must become active in an environment almost devoid of oxygen, and further, burrow actively into the gut wall to molt and continue its life cycle. With such a wide diversity of activity and habitat, the ideal answer is a minimal oxygen requirement.

On the basis of these results it is probably safe to conclude that one of the adaptations necessary for parasitic life is an adjustment of oxygen requirements. These data may also indicate that one method of acquisition of the parasitic habit is by means of such an adjustment since closely related species of worms are capable of living in different habitats partially at least by virtue of their adjustments to varying oxygen conditions. We may further speculate that as the physiological characteristics of worm populations gradually alter, new parasites will evolve and old ones will extend their host ranges.

SUMMARY

Oxygen consumption relative to oxygen tension was determined for three nematodes. Tensions ranged from 20.7 to 383.1 mm Hg. Survival times relative to oxygen tension were determined for two nematodes.

Rhabditis elegans, a free-living nematode increased consumption with tension until a tension of about 121.5 mm Hg was reached. Beyond this point further increases had little effect upon consumption.

Rhabditis strongyloides, a nematode recovered from skin lesions on a cow increased consumption with tension until a tension of 58.5 mm Hg was reached. Beyond this point increases had little effect on consumption.

Small horse strongyle larvae, free-living stages of a parasitic form, consumed oxygen at a very low and almost constant level over the entire range of oxygen tensions used.

The survival times of *Rhabditis strongyloides* and *Rhabditella axei* increased linearly with the oxygen tension.

The adaptive significance of the work reported is discussed.

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TWO NEW SPECIES OF *KLOSSIA* (SPOROZOA: ADELEIDAE) FROM A DEER MOUSE AND A BAT

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During the summer of 1954 one of us (F. J. K.) took part as parasitologist in a collecting expedition to Grand Canyon National Park, Arizona. Others on the expedition were mammalogists under the leadership of Dr. Donald F. Hoffmeister, curator of the University of Illinois Natural History Museum. Parasites were collected from mammals trapped by members of the expedition, and fecal samples from some of the animals were placed in 2.5% potassium bichromate solution and brought back to Urbana for examination. The samples were mixed thoroughly, placed in a thin layer in Petri dishes for a week to sporulate, and stored in a refrigerator. They were examined microscopically after sugar flotation, using a Leitz Ortholux microscope with apochromatic objectives.

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The present paper describes two new species of *Klossia* (SPOROZOA: ADELEIDAE) from oocysts found in the feces of a deer mouse, *Peromyscus maniculatus*, and a western big-eared bat, *Corynorhinus rafinesquii*. Measurements in the descriptions and the discussions are in microns.

Klossia perplexens n. sp.

Diagnosis: Klossia: Sporulated oocysts (Fig. 1) ellipsoidal, with smooth wall composed of

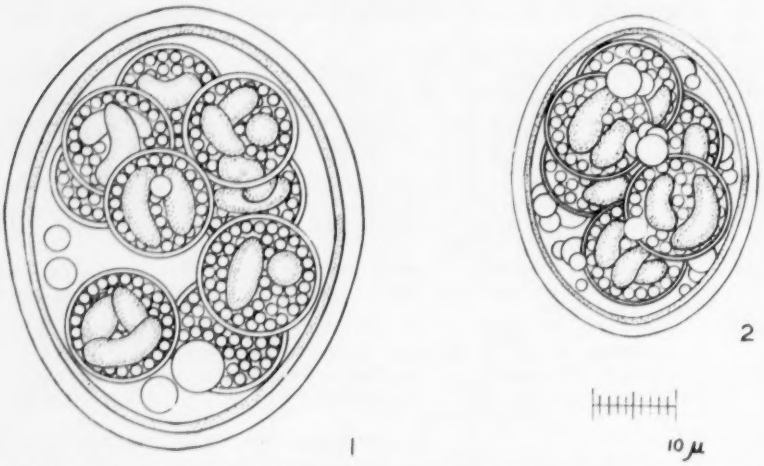

FIG. 1. Oocyst of *Klossia perplexens* n. sp. from *Peromyscus maniculatus*, optical section, × 1300. Only 9 of the 12 sporocysts are shown.

FIG. 2. Oocyst of *Klossia variabilis* n. sp. from *Corynorhinus refinesqii*, optical section, × 1300. Only 6 of the 8 sporocysts in this oocyst are shown.

two layers. Outer layer colorless, transparent, about 2 thick. Inner wall pale brown, 0.5 thick. Micropyle absent. Seven sporulated oocysts 35.5–43.7 by 42.2–53.0, mean 39.9 by 47.9. Standard deviation of length, 3.53, of width 2.99. Length-width ratio ranging from 1.17 to 1.22, mean of 1.20, standard deviation 0.018. Oocysts lacking refractile granule and residual body, but scattered among sporocysts, about a dozen round, colorless, homogeneous globules 3 to 6 in diameter, resembling carbohydrate or fat globules, chemical tests not run. Twelve sporocysts in each of 4 oocysts carefully counted, apparently same number in other 3. Sporocysts packed so tightly in oocysts that counting them is difficult. Sporocysts spherical, wall colorless, 0.5 thick. Stieda body lacking. Eight sporocysts from 2 oocysts ranged in diameter from 12.9 to 14.2, mean 13.3. Each sporocyst containing 4 sausage-shaped, colorless sporozoites, arranged in no particular order. Colorless, spherical granules about 1.2 to 1.5 in diameter in constant Brownian movement filling remaining space in sporocysts. These granules very numerous, preventing full visibility of all sporozoites. One or two larger granules about 3 in diameter similar to those in oocysts sometimes present in sporocysts.

K. perplexens was found in the feces of a deer mouse, *Peromyscus maniculatus*, collected July 10, 1954 by Mr. K. R. Nelson on the west side of Cedar Mountain, which is situated on the south rim of Grand Canyon.

Klossia variabilis n. sp.

Diagnosis: Klossia: Sporulated oocysts (Fig. 2) ellipsoidal, with smooth two-layered wall. Outer layer colorless, transparent, about 2 thick. Inner wall pale brown, 0.5 thick. Micropyle lacking. Several dozen round, colorless, homogeneous gobules of residual matter scattered among sporocysts of each oocyst. Refractile granule absent. Number of sporocysts variable, ranging from 4 to 13, mean 8.5, mode 8, in 30 carefully-studied oocysts (Table I). Oocysts measured

22.2–36.4 by 27.9–48.0, mean 30.4 by 39.3, standard deviation of length 4.13, of width 3.06. Length-width ratios ranged from 1.20 to 1.42, mean 1.29, standard deviation 0.035. In general, the more sporocysts present, the larger the oocyst. Oocyst size ranged from 22.2 by 27.9 for oocyst containing 4 sporocysts to 36.4 by 48.0 for one containing 13 sporocysts (Table 1).

Sporocysts resemble those of *K. perplexens*, being spherical, with colorless wall about 0.5 thick and without a Stieda body; average diameter about 13. Each sporocyst containing four sausage-shaped, colorless sporozoites lying in no particular order; space between sporozoites filled with colorless, spherical granules about 1.2 to 1.5 in diameter in constant Brownian movement. Some sporocysts containing also a few larger colorless granules.

Host: Western Big-eared Bat, *Corynorhinus rafinesqii* (in feces).

Locality: Hearst Ranch, south rim of Grand Canyon.

Collected by: Dr. Hoffmeister, July 8, 1954.

An attempt was made to infect baby white rats (*Rattus norvegicus*) with *K. variabilis* oocysts. On October 8, 1954, three 3-day-old rats in one litter and four 2-week-old rats in another were fed at least 20 sporulated oocysts each by pipette. Fecal examinations were made on the exposed rats and on their litter-mates before exposure and periodically for 23 days after exposure. No oocysts were ever seen.

DISCUSSION

In classifying the COCCIDIA, the characters of both the cysts and the developmental stages in the host are used. However, in many cases, as in the present one, nothing

TABLE I.—*Relation of number of sporocysts to size of oocysts in* Klossia variabilis *from* Corynorhinus rafinesquii

Sporocyst Number	Number of Oocysts	Oocyst dimensions in microns		Oocyst length-wide ratio	
		Range	Mean	Range	Mean
4	1		22.2 by 27.9		1.26
5	1		26.2 by 32.4		1.24
6	1		31.5 by 37.7		1.20
7	2*		28.4 by 38.2		1.35
8	12*	27.0–31.5 by 34.2–42.2	29.1 by 38.1	1.2–1.4	1.31
9	5**	31.1–33.7 by 40.0–41.3	32.7 by 40.6	1.2–1.3	1.24
10	5	31.1–34.2 by 38.6–43.9	32.8 by 42.0	1.2–1.3	1.28
11	2	32.4–33.3 by 43.5–44.4	32.8 by 44.0		1.34
12	0				
13	1		36.4 by 48.0		1.32
Mean 8.5 of Total	30	22.2–36.4 by 27.9–48.0	30.4 by 39.3	1.2–1.4	1.29

* 1 oocyst was shrunken and could not be measured.
** 1 oocyst was shrunken and another was on end; these could not be measured.

is known about the parasitic stages so that one must depend entirely on the cysts. Of the eight genera whose sporocysts contain four sporozoites each, there is one sporocyst per oocyst in *Mantonella*, two in *Isospora*, three in *Chagasella*, four in *Wenyonella*, 16 in *Pythonella*, and variable numbers in *Klossia*, *Orcheobius* and *Cariniella*. The present species would fall into one of the last three genera, unless they should turn out to belong to a new genus. These genera are at present usually assigned to the family ADELEIDAE.

The taxonomy of the COCCIDIA has been discussed by Wenyon (1926), Grassé (1953), Hall (1953), Reichenow (1953) and Kudo (1954), no two of whom are in agreement. All, incidentally, omit mention of the genera *Cariniella* Pinto, 1926 and *Pythonella* Ray and Das Gupta, 1937.

The genus *Klossia* has been reviewed by Nabih (1938). *Klossia helicina* A. Schneider, 1875 is a common parasite of land snails of the genera *Helix* and *Succinea* in Europe, but has apparently not been reported from the western hemi-

sphere. It was first described, but not named, by Kloss (1855). Its oocysts are spherical, average 75 microns or more in diameter, and contain 40–160 spherical sporocysts. The oocysts have no micropyle and contain no residual body. They have a wall about 2 microns thick composed of two layers. The sporocysts average 12–13 microns in diameter, and contain a residual body. (According to Nabih the sporocysts have no residual body on elimination.) They have neither a Stieda body nor a micropyle.

K. soror A. Schneider, 1881 was described from the kidney epithelium of the snail, *Neritina* (= *Theodoxas*) *fluviatilis*, in France. Its oocysts are spherical to ovoid, and are 40 to 60 microns in diameter. The sporocysts are spherical; Schneider stated merely that they are numerous, but showed 36 in his figure. The oocysts have no micropyle, but they do have a residual body. The oocyst wall is composed of two layers. The sporocysts average 7.6 microns in diameter, and have a residual body but no Stieda body or micropyle.

K. loossi Nabih, 1938 was described from the kidney epithelium of slugs of the genera *Arion* and *Limax* in Germany. Its oocysts are spherical, and average less than 50 microns in diameter. They have no residual body or micropyle. The oocyst wall is composed of two layers. The oocysts contain less than 36 (usually 32) spherical sporocysts averaging 16 microns in diameter. The sporocysts have no Stieda body or micropyle; they have some residual granules around their periphery.

K. vitrina Moroff, 1911 was described from the kidney epithelial cells of the snail, *Vitrina elliptica*, in Germany. No ripe oocysts were seen. About 16 sporozoite nuclei were formed by the zygote, and 4 sporozoite nuclei were seen in each sporoblast. Without further investigation, it would be difficult to say whether this is actually a distinct species or a synonym of *K. helicina*, as a number of later writers have thought (cf. Wenyon, 1926, and Nabih, 1938).

Chatton (1937) mentioned a *Klossia helicina* in the kidney of the marine snail, *Loripes lacteus*, but did not describe it. It may well be a separate species, rather than *K. helicina*.

The genus *Orcheobius* was established by Schuberg and Kunze (1906) for *O. herpobdellae*, which they described from the epithelial cells of the testis of the leech, *Herpobdella atomaria* (*Nephelis vulgaris*) in Germany. This genus differs from *Klossia* in that its macrogametocytes are elongate, while those of *Klossia* are spherical. The oocysts of *O. herpobdellae* are ellipsoidal, and have a single-layered wall without a micropyle. They do not contain a residual body. They contain 25 to 30 spherical sporocysts, each containing four sausage-shaped sporozoites and a large residual body, but no Stieda body. While no dimensions were given by Schuberg and Kunze, it can be estimated from Kunze's (1907) illustration that the oocysts measured about 70 by 100 microns, and the sporocysts about 22 microns in diameter.

The genus *Cariniella* was established in the subfamily KLOSSINAE by Pinto (1926) for *C. carinii*, which he described from the feces of a frog, *Leptodactylus ocellatus*, in Brazil. No intracellular stages were found in sections from the frog, and Pinto suggested that perhaps this species might be a parasite of some animal the frog had eaten. The oocysts are ellipsoidal, and measure 62 by 172 microns. They have a single, very thin wall, no micropyle, and no residual body. They con-

tain a large number of ellipsoidal sporocysts. Pinto gave no numbers, but judging from his illustration there must be well over 100 sporocysts in each oocyst. The sporocysts average 10.3 by 17.2 microns, have a micropyle at each end, and contain 4 sporozoites and a residual body.

C. cruzi (Carini and Pinto, 1926) was described from the region corresponding to the genital apparatus of an earthworm (no scientific name given) in São Paulo, Brazil. It was originally assigned to the genus *Orcheobius*, but transferred to *Carinella* by Pinto (1926) because of the shape of its sporocysts. The oocysts are ovoid, with mean measurements of 38 by 52 microns. The oocyst wall is one layer thick, and has a micropyle with protruding bilobed lips. It contains a residual body and 12 ellipsoidal sporocysts, each with 4 sporozoites lying lengthwise in it and a large residual body. The sporocysts measure 10 by 17.2 microns, and, as illustrated, have a clear knob at each end. These were considered micropyles by Pinto (1926), but might be Stieda bodies.

Finally, Becker and Zimmerman (1954) once found oocysts containing 8, 12 or 16 sporocysts in an Iowa farm chicken. The oocysts with 16 sporocysts measured 34.4 by 41.8 microns and those with 8 sporocysts measured 27.6 by 34.0 microns; no oocysts with 12 sporocysts were measured. The oocyst wall was comparatively thin. The sporocysts were subspherical, those in the oocysts with 16 sporocysts averaging 11.0 by 11.3 microns, and those in the oocysts with 8 sporocysts averaging 10.3 by 11.0 microns. There appeared to be 4 sporozoites in each sporocyst, but they were too tightly packed to be certain. Becker and Zimmerman (1954) attempted to infect chickens with these oocysts without success, and were of the opinion that they may have simply been passing through the chicken after having been eaten.

Cariniella differs from *Klossia* and *Orcheobius* in the morphology of its sporocysts, which are elongate and have a micropyle at each end. The oocysts of *Klossia* and *Orcheobius* look alike; both have a variable number of spherical sporocysts each with 4 sporozoites. These genera differ in the shape of their macrogametocytes, *Klossia* having spherical ones, and *Orcheobius*, elongate. The oocyst wall of *Klossia* is composed of two layers, and that of *Orcheobius* of one, but this is a variable feature among the COCCIDIA, and sometimes cannot be determined from published descriptions. Whether these are sufficient differences to justify separation into two genera is open to question, but further work will be needed before the question can be answered.

Since our species are described only from oocysts, they are assigned to the genus *Klossia* partly because they seem to resemble it more than any other genus and partly as a matter of practical convenience. Later investigations may show that a new genus should be established for them, but this would be unwise at present.

K. perplexens and *K. variabilis* differ from all species of *Klossia* and *Orcheobius* in the number of sporocysts in their oocysts. *K. variabilis* has 4 to 13 sporocysts, with an average of 8.5, while *K. perplexens* has 12. *K. helicina* has 40 to 160; *K. soror*, about 36; *K. loossi*, usually 32; and *O. herpobdellae*, 25 to 30. The oocysts found by Becker and Zimmerman (1954) in the chicken had 8 to 16 sporocysts, but both these oocysts and their sporocysts were somewhat smaller than ours.

The oocyst wall of *K. helicina*, *K. soror*, *K. loossi*, *K. perplexens* and *K. variabilis* is composed of two layers; that of *O. herpobdellae* is composed of one. The

oocysts of *Klossia* from snails do not pass out of the host intact. They are relatively thin-walled, and rupture and release the sporocysts in the kidney canaliculi. In *K. perplexens* and *K. variabilis*, however, intact, thin-walled oocysts were found in the feces. The oocysts found by Becker and Zimmerman (1954) in the chicken were comparatively thin-walled, but intact.

All species of *Klossia* heretofore described have been found in snails or slugs in Europe. The single species of *Orcheobius* was found in a leech in Europe. Nothing is known about the incidence, or even the existence, of these COCCIDIA in American molluscs and annelids.

The morphological differences between the forms from the deer mouse and the bat are sufficient to justify separation into two species. The probability is extremely small that in a series of 34 oocysts from two different hosts, all four from one host would have 12 sporocysts and all 30 from the other host would have 4 to 13, but never 12.

A significant difference was found in size of oocysts between the two species. Using the t test, the differences in both oocyst length and width were significant beyond the 1% level ($t = 3.73$ for length and 4.45 for width). Even when only the eight *K. variabilis* oocysts containing 10 to 13 sporocysts (*i.e.*, those most likely to be the same size as the *K. perplexens* oocysts) were used, the differences in both length and width were significant at the 1% level ($t = 2.84$ for length and 3.01 for width).

It is possible that *K. perplexens* and *K. variabilis* may actually be parasites of invertebrates eaten by the deer mouse and bat. Further work must be done to eliminate or establish this possibility. There is little chance, however, that they are parasites of snails, slugs or leeches. The western big-eared bat is entirely insectivorous, capturing its prey in flight. The deer mouse eats insects and other arthropods in addition to vegetable materials. However, Cedar Mountain, where it was captured, is largely arid rock and sand, with little vegetation; molluscs and leeches probably do not exist there.

SUMMARY

Two new species of *Klossia* (SPOROZOA; ADELEIDAE) are described from oocysts in the feces of mammals from Grand Canyon National Park, Arizona. The oocysts of *K. perplexens* n. sp. from the deer mouse, *Peromyscus maniculatus*, measure 39.9 by 47.9 microns and contain 12 spherical sporocysts 13.3 microns in diameter. The oocysts of *K. variabilis* n. sp. from the western big-eared bat, *Corynorhinus rafinesquii*, average 30.4 by 39.3 microns and contain 4 to 13 (average 8.5) spherical sporocysts. An unsuccessful attempt was made to infect baby white rats with *K. variabilis*.

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A TRYPANOSOME FROM THE BIG BROWN BAT, *EPTESICUS FUSCUS FUSCUS* (BEAUVOIS), IN MINNESOTA

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Trypanosomes have been reported or described from about 50 species of CHIROPTERA throughout most of the temperate and tropical parts of the world. Comparatively few bats in this country have been reported infected with trypanosomes, and, as far as the author is aware, no detailed descriptions of the trypanosomes of U. S. bats are available.

Wood (1952) gave a summary of the bats of southwestern United States reported infected with trypanosomes. These include *Antrozous pallidus pacificus*, *Myotis occultus*, and *Eptesicus fuscus bernardinus* in California, *Myotis velifer velifer* in Arizona, and *Pipistrellus hesperus maximus* in Texas. The only additional published report from the United States is that of Tromba (1951) of a trypanosome from *Eptesicus fuscus fuscus* in Virginia.

The bat trypanosomes have attracted considerable attention since some workers have considered certain ones to be identical to *Trypanosoma cruzi* Chagas, (Clark and Dunn, 1932). Also species of *Desmodus*, the vampire bats, have been incriminated in the transmission of *Trypanosoma equinum* (Elmassiani) and *Trypanosoma hippicum* Darling to horses and other domestic animals, occasionally becoming infected themselves (Acosta and Romaña, 1938; Johnson, 1936).

During the winter of 1951, *Eptesicus fuscus fuscus* from sandstone caves along the banks of the Mississippi River in St. Paul were found infected with a species of trypanosome, similar in form to *Trypanosoma cruzi*. During the rest of 1951 and for the following two winters, 79 bats of this species were collected from various sandstone caves in and around St. Paul and Stillwater, Minnesota. Seventeen

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(21.5 percent) of these were infected. Also, during the summer of 1953, one Red Bat, *Lasiurus borealis*, was found to contain trypanosomes similar to those of *E. f. fuscus*. Microfilariae, also, were occasionally encountered in the blood of *E. f. fuscus*.

The following account is a description of the trypanosome, incorporating morphology, infectivity to laboratory animals, and presence or absence of tissue stages as diagnostic characters.

The author wishes to acknowledge the assistance of all who helped in this study, particularly Mr. John C. Holmes who accompanied the author in all of the field work involved and who assisted in various details of the laboratory work. Dr. Franklin G. Wallace of the Department of Zoology at the University of Minnesota, where this study was done, was a constant advisor to the project, and aided in the preparation of the manuscript. Acknowledgements are also due to Dr. Asa C. Chandler of the Department of Biology at the Rice Institute who was of assistance in the preparation of the manuscript.

MATERIALS AND METHODS

Diagnosis of infection was accomplished by examination of thick and thin blood films and fresh preparations made from the blood of a clipped toe, and by inoculation of SNB-9 culture medium (Diamond and Herman, 1954). Cultures were inoculated with heart blood, drawn with a hypodermic syringe. The classical NNN medium and Kelser's medium were tried also, but did not give as satisfactory results.

Measurements of the trypanosomes were made on camera lucida drawings with the aid of a calipers. Care was taken in the measurement of body length (posterior end to base of free flagellum), center of nucleus to anterior or posterior ends, and flagellar length to include all undulations. Measurements on the body of the trypanosomes were made along the approximate mid-line.

Animals used in infection experiments—rats, mice, hamsters, and rabbits—were laboratory-reared and were known not to have previous trypanosome infections. In all cases the animals were inoculated within several days after weaning. Inoculations were by intraperitoneal injections of trypanosome culture or, in rabbits, intravenous injections of the blood of an infected bat. In several cases, intraperitoneal injections of cortisone were given previous to, or simultaneous with, inoculation of the cultured parasites in an attempt to lower the animal's resistance. Diagnosis of infection was accomplished by means of thick blood films, fresh blood preparations, or, in the case of the mouse experiments, by inoculation of SNB-9 culture medium.

The hearts, livers, kidneys, spleens, stomachs, lungs, and intestines from three infected bats, fixed in Bouin's or Zenker's fluids, were sectioned at 7 or 8 microns, stained with a mixture of 1.25 cc. of Giemsa stain, 1.5 cc. of methyl alcohol, and 50 cc. of distilled water at a pH of 7.0 and mounted in piccolyte or balsam after dehydration in tertiary butyl alcohol.

Nine laboratory-reared *Aedes aegypti* Linn. were fed on a cotton swab which had been dipped in trypanosome culture. The mosquitos were then maintained on a diet of boiled raisins until they were examined for trypanosomes. The entire gut, salivary glands, and Malpighian tubules were examined.

MORPHOLOGY

The trypanosome is monomorphic, similar in size and proportions to *Trypanosoma cruzi*, with a large kinetoplast located close to the finely tapered posterior end. The undulating membrane is only slightly convoluted and the free flagellum is generally slightly greater than half of the body length. The nucleus is usually situated about a third of the body length from the anterior end. Metachromatic granules are present just anterior to the nucleus in Giemsa-stained specimens. No evidence of multiplication in the blood stream was observed.

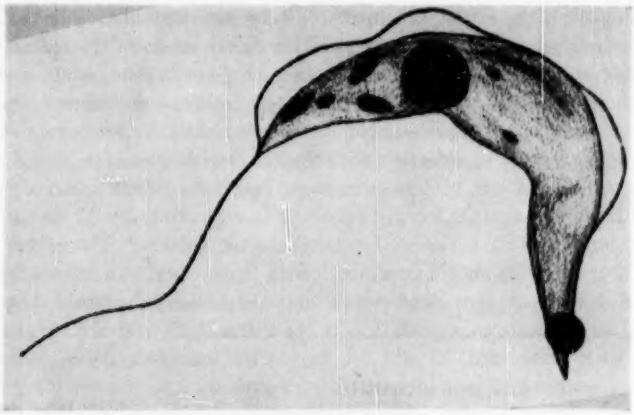

FIG. 1. *Trypanosoma* sp. from *Eptesicus fuscus fuscus* (Beauvois). Camera lucida drawing. ×970.

Measurements of 38 trypanosomes are presented in the following table.

TABLE I.—*Measurements of 38 trypanosomes from the bat,* Eptesicus f. fuscus

Measurements*	Mean	Standard deviation	Range
Total length	17.8	1.5	14.8–21.1
Body length	11.2	1.4	9.0–14.3
Free flagellum	6.6	1.0	4.6– 8.7
Center of nucleus to kinetoplast	5.6	1.2	3.5– 9.5
Center of nucleus to anterior end	4.3	1.3	1.4– 7.5
Center of nucleus to posterior end	6.9	1.3	4.6– 9.5
Center of kinetoplast to post. end	1.0	0.3	0.0– 3.0
Body width (at nucleus)	1.5	0.1	0.8– 2.7
Diameter of kinetoplast	0.5	0.2	0.2– 0.7
Nuclear index**	1.87	1.20	0.61– 6.79

* The measurements are given in microns.
** The distance from the center of the nucleus to the posterior end divided by the distance from the center of the nucleus to the anterior end.

As indicated by the standard deviations, the measurements are considerably variable. The large standard deviation of the nuclear index is primarily due to three trypanosomes which had their nuclei placed very close to the anterior end (nuclear indices of 3.76, 5.93, and 6.79).

INFECTIVITY TO LABORATORY ANIMALS

Attempts at infecting laboratory animals with the bat trypanosome were unsuccessful. Three rats were given 0.4, 0.3, and 0.3 cc. of trypanosome culture intraperitoneally and were examined by means of thick blood films and fresh blood

preparations every second or third day from 6 to 28 days after inoculation. All remained negative. Four mice were given 0.2 cc. of trypanosome culture intraperitoneally and then examined by the culture method 10 days after inoculation and, except for one mouse which died shortly after the first examination, every second or third day thereafter until 30 days had elapsed. All examinations of mice gave negative results.

One hamster was given 0.3 cc. of culture alone intraperitoneally, while two other hamsters were given 0.4 cc. and 0.5 cc. preceded by 0.3 and 0.4 cc. of cortisone respectively. These were examined by means of thick blood films every second or third day from the 5th or 6th to the 28th or 30th day after inoculation. After termination of the observation period, cultures were made of the spleen, liver, and heart of one animal along with blood smears of these organs, while a culture was made of the spleen only of one other, and blood smears were made from the heart, lungs, liver, kidney, and spleen. No further examination was made of the third hamster. No evidence of infection with the bat trypanosome was found.

Also inoculations into rabbits gave negative results. One rabbit was given 1.0 cc. of cortisone and again 0.5 cc. of cortisone intraperitoneally 12 and 10 days preceding inoculation with 1.0 cc. of bat trypanosome culture. The rabbit was killed 16 days after inoculation, but examination of thick blood smears made 10 and 16 days after inoculation showed no signs of trypanosome infection. Another rabbit inoculated at the same time with 0.95 cc. of culture only did not show evidence of infection when examined 10 and 16 days after inoculation. Five-tenths cc. of infected bat heart blood was inoculated intravenously into the marginal ear vein of each of two rabbits. These were examined by thick and thin blood films and by fresh blood preparations every second or third day from 10 to 28 days after inoculation, but no trypanosomes were found.

TISSUE EXAMINATION

A careful microscopic examination of sections of heart, liver, kidneys, spleen stomach and intestine of one bat was made in an effort to locate tissue stages of the trypanosome. The same organs and the lungs from two other infected bats were given a more cursory examination. No tissue stages were found.

VECTOR EXPERIMENTS

Examination of the alimentary tract of several of the *Aedes aegypti* mosquitos 1 day, 3 days, 8 days and 12 days after feeding gave no evidence that the trypanosome would develop in this insect.

DISCUSSION

It has become increasingly apparent that morphology is not always the most accurate guide to a natural classification of organisms. The trypanosome described here from *Eptesicus fuscus fuscus* in Minnesota agrees in morphology with the descriptions of trypanosomes from *Pipistrellus pipistrellus* in England reported by Coles (1914), from *Hemiderma perspicillatum* in Venezuela by Dias and Pifano (1941), and from *Carollia perspicillata* in Brazil by Dias (1940).

Unfortunately, many earlier descriptions of bat trypanosomes are not sufficient

for comparison with the present trypanosome. Battaglia (1904), in describing the species *Trypanosoma vespertilionis* from *Nyctalus noctula* (then known as *Vesperugo noctula*), gave only the body length and body width.

The trypanosome of *Hemiderma perspicillatum* of Venezuela (Dias and Pifano, 1941) was found to be infective to laboratory rats, mice, guinea pigs, dogs and an opossum, besides several other species of bats, and to develop into the leishmania form in the tissues of the bat. The form from *Carollia perspicillata*, although almost identical to the former in morphology, was found to be infective to laboratory rats and guinea pigs, but not to have tissue stages in the bat (Dias, 1940). The present trypanosome was not infective to laboratory animals nor could tissue stages of the parasite be found. However, Huff and Christian (1955) observed a similar trypanosome in *Eptesicus fuscus* from a colony in the vicinity of Washington, D. C. The organism was seen in the living state in fresh blood, in stained smears, and was isolated in diphasic blood agar. Leishmaniform bodies similar to those in *Trypanosoma cruzi* were observed in sections of a bat from the same colony. They note that Romaña (1944) recorded a similar infection in Argentine bats of the genus *Eptesicus*.

It is apparent, therefore, that trypanosomes nearly identical in morphology are not necessarily identical in infectivity to laboratory animals and life cycle, and should not be considered the same species on morphological characters alone. It is evident that a more extensive survey of the literature is necessary in order to establish the identity of this trypanosome.

SUMMARY

A trypanosome found infecting Minnesota bats of the species *Eptesicus fuscus fuscus* (Beauvois) is described. Morphologically, it is similar in size and proportions to *Trypanosoma cruzi*. Attempts to infect laboratory rats, mice, hamsters, and rabbits were unsuccessful. Histological examination of the heart, liver, kidneys, spleen, lungs, stomach, and intestine of three infected bats did not reveal tissue stages of the parasite. Development of the trypanosome was not observed in *Aedes aegypti* which had fed on trypanosome culture.

The inadequacy of morphological characters in allowing differentiation of separate trypanosome species is stressed, and for this reason a more extensive survey of the literature is necessary in order to establish the identity of this trypanosome.

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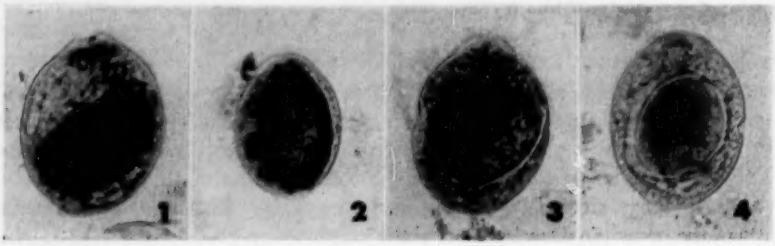
RESEARCH NOTES

INGESTION OF *TRICHURIS TRICHIURA* OVA BY *BALANTIDIUM COLI*

While examining a stool specimen containing cysts and trophozoites of *Balantidium coli*, a number of the trophozoites were found to contain ova of *Trichuris trichiura*. As ingested matter of this size had never been encountered by us in previous stools containing this ciliate, reports of this activity were sought in the literature. Kourí (In Vol. III of Gradwohl's "Clinical Laboratory Methods and Diagnosis," 1948) reported one case in which *B. coli* showed from one to four ingested *Trichuris* ova.

In our stool specimen approximately one percent of the ciliates had ingested *Trichuris* ova. Although the stool specimen contained a few ova of hookworm and *Heterodera marioni*, none of these eggs were found in *Balantidium*. The unstained, formalin-preserved balantidia ranged from 52 to 84 microns long by 43 to 65 microns wide, with a mean of 65 by 50 microns. Thus, they were approximately the length of hookworm ova and not much longer than the ingested *Trichuris* ova.

All figures were made from hematoxylin-stained slides, and are shown at a magnification of 400 times. The typical appearance of a *Trichuris* egg in *B. coli* is shown in Figure 1. Some



of the smaller balantidia were scarcely longer than the ingested egg (Fig. 2). Ova apparently moved around within the ciliate, as they could be found in longitudinal, diagonal and horizontal positions. The majority of balantidia exhibited newly ingested ova, but some (Fig. 3) contained only the shell, as though the egg had been in the ciliate long enough for digestion of the egg contents to have occurred.

Kourí apparently found a larger race of *Balantidium* in his stool specimen, for, in comparison with the ova of *Trichuris*, the ciliates appeared to average about 100 by 80 microns, about 50 percent larger than those in our case. None of ours showed more than one ingested egg and the ingestion of a second egg would have been difficult. Kourí also stated that the balantidia would ingest the eggs and after a few minutes would expel them. The balantidia with ingested eggs were not found by us until after the material had been preserved hence the actual process of ingestion was not observed. Apparently all eggs were not immediately expelled, for some appeared to have been digested.

Occasionally a *Balantidium* had ingested another *Balantidium* (Fig. 4), a condition previously reported (McDonald, 1922, Univ. Calif. Pub. Zool. **20**: 243–300; Nelson, 1933, Am. J. Hyg. **18**: 185–201), but not illustrated.

A list of items found within *Balantidium*, as reported by various workers, include leucocytes, erythrocytes, tissue cells, bacteria, yeast, *Blastocystis*, starch granules, various intestinal Protozoa (including *Balantidium*) and ova of *Trichuris*. Perhaps other small ova are ingested when available. *B. coli*, therefore, appears to be capable of ingesting any potential food item, even some almost as large as itself.—GERALD E. KLINK AND ROBERT B. BURROWS, Lt. Col., MSC, *Sixth Army Area Medical Laboratory, Fort Baker, Calif.*

TWO CASES OF PHYSALOPTERA INFECTION IN MAN FROM COLOMBIA

Recently, during the routine examination of feces for parasitic infection in the diagnostic laboratory, Clinica de la Nutrición, Bogotá, Colombia, unfamiliar helminth eggs were detected in two unrelated patients. The first was an eight year old boy who had never been away from Bogotá, the second was an adult male who had traveled only within Colombia. Preserved specimens were sent to the Department of Tropical Public Health, Harvard School of Public Health, where they were identified as eggs of *Physaloptera* sp. by the junior author who had previously obtained specimens of *Physaloptera* from a cat in Japan and had preserved specimens with which the Colombian eggs were compared. The eggs are oval with thick translucent shells and are embryonated (Fig. 1.).

The boy received a single treatment with 0.8 gm. Crystoids anthelmintic (Caprokol). Examination of his stools for worms were not made following treatment. However, no *Physaloptera* eggs were found in the stools on the 4th and 30th days after treatment. The adult male patient voluntarily left the hospital without treatment, and further contact with him could not be made.

Since worms were not obtained, specific identification of the parasite could not be established. Further, it is not known from the evidence at hand whether these were cases of spurious parasitism, or whether the single treatment with Crystoids effectively removed the parasites in the one case. Neither patient presented symptoms which could be associated with the parasite.

Thus far, only one species, *Physaloptera caucasica* v. Linstow, 1902, has been reported from man. This species has been recorded from Southern Russia by von Linstow (1902, Centralbl. Bakteriol. 1. Abt. Orig. 31: 768–771) and Tropical Africa by Leiper (1907, Tr. Soc. Trop. Med. and Hyg. 1: 76–82; 1913 ibid 6: 265–297), Ortlepp (1926, J. Helm. 4: 199–202) and Blackie (1932, No. 5, Memoir Series London School of Hygiene and Tropical Medicine,

FIG. 1. Egg of *Physaloptera* sp. from a human case in Colombia. ×400.

91 pp.). It is stated by Faust (1949, Human Helminthology, A Manual for Physicians, Sanitarians and Medical Zoologists, 3 Ed. 744 pp., Philadelphia) and Craig and Faust (1951, Clinical Parasitology. 5 Ed. 1032 pp., Philadelphia) that Faust and Martinez (1935, J. Parasit. 21: 332–336) found *Physaloptera* eggs in the feces of a native of Panama. A careful review of this publication by Faust and Martinez failed to reveal any mention of *Physaloptera*, although eggs "identical with *Gongylonema pulchrum*" are recorded from an adult male patient, a resident of the village of Gatuncillo. It would appear therefore that the present finding of *Physaloptera* eggs in two Colombian patients constitutes the first such observation in the Americas.—ANDRÉS SORIANO LLERAS, *Clinica de la Nutrición, Bogotá, Colombia,* and CHIATUNG PAN, *Department of Tropical Public Health, Harvard School of Public Health, Boston, Massachusetts.*

ARTIFICIAL FEEDING OF CERTAIN ECTOPARASITES THROUGH MEMBRANES

The method is an adaptation of that used by Fuller *et al.* (1949, Pub. Health Rep., U. S. Pub. Health Serv. **64**: 1287). Membranes used were three-week-old mouse skin, four-week-old chick skin, and two-day-old turkey poult skin. The last was used to replace newly hatched chick skin, which was unavailable at the time. Skins were kept frozen until used, and were untreated except for clipping of down or hair.

Insects tested were the sheep ked (*Melophagus ovinus* (L.)), the hog louse (*Haematopinus suis* (L.)), cattle lice (*H. eurysternus* (Nitz.)) and *Linognathus vituli* (L.)), and the dog louse (*L. setosus* (von Olfers)).

The results of the feeding trials are shown in Table I. In all cases, putrefaction of the membrane after continued use did not repel the insects or affect their ability to penetrate it. However, these insects did not attempt to feed unless the medium was warmed to approximately

TABLE I.—*Percentages of various insects feeding to repletion on sheep blood through three animal skin membranes*

	M. ovinus	*L. vituli*	*L. setosus*	*H. suis*	*H. eurysternus*
Approximate no. insects used in testing each membrane	10	20	20	10	20
3-wk.-old mouse skin, hair clipped	50	50	10	50	10
4-wk.-old chick skin (down not present)	100	0	0	100	0
2-day-old turkey skin, down clipped	100	10	10	100	100

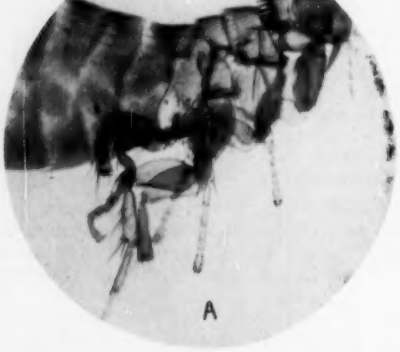
36° C. Newly emerged sheep keds fed just as well as old adults taken from sheep. All stages of *H. suis* fed equally well. Only second-instar nymphs and adults of *H. eurysternus, L. vituli* and *L. setosus* were adequately tested.

The blunt tarsal claws of *L. vituli, L. setosus* and *H. eurysternus* prevented them from obtaining footholds on skins devoid of down or hair. For these species it was necessary to leave some cover on the membrane.

In newly emerged sheep keds there is a period of about 24 to 36 hours during which they will not feed. Moreover, older keds feed regularly only about every 36 hours when on the sheep; when they are kept at 30° C. in the laboratory, they will take a blood meal with about the same frequency (unpublished data). *H. suis* feeds once about every 24 hours in the laboratory, whereas *H. eurysternus* feeds every 8 hours. This rather constant feeding periodicity shown by *M. ovinus, H. suis* and *H. eurysternus* explains the frequent refusal of these species to feed when nothing was known of the time at which their previous blood meal was taken. *L. vituli* and *L. setosus* appear to feed almost continuously on laboratory animals; at least they remain attached continuously and void feces regularly. In the case of the last two species refusal to attach to the membrane did appear to indicate its unsuitability. The feeding frequency of the lice on their normal hosts was not investigated. (Contribution No. 3314, Entomology Division, Science Service, Department of Agriculture, Ottawa, Canada).—W. A. NELSON, *Veterinary and Medical Entomology Section, Science Service Laboratory, Lethbridge, Alberta.*

AN EIGHT-LEGGED FLEA, *ORCHOPEAS HOWARDI HOWARDI* (BAKER)

A number of anomalous flea specimens have been described, most of which show peculiar variations in the shape of the reproductive structures. An eight-legged specimen of *Orchopeas howardi howardi* (Baker) was found in a lot of fleas collected in Ithaca, N. Y., July 3, 1950. The fleas in this lot had been collected from a gray squirrel, *Sciurus carolinensis leucotis* (Gapper) brought to us by R. M. Roecker.



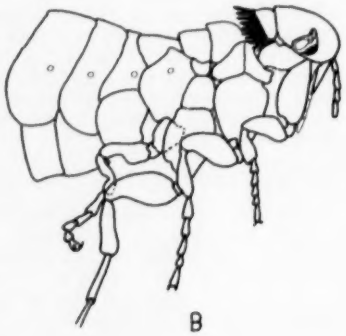


FIG. A. Photograph showing all eight legs of the flea.

FIG. B. Line drawing showing the four legs on the right side in their proper relation to each other.

The flea, as can be seen from the photograph figure A and from the outline drawing figure B, has eight legs. The pro-meso- and metathoracic legs are quite normal, but in addition there is a fourth pair of legs attached to the metathoracic segment somewhat reduced in size and also slightly distorted. The coxae of this pair are posterior to the metathoracic legs and seem to be attached at the posterior margin of the metepimeron. The tarsal segments are missing from the left fourth leg but are all present on the right leg.

The tarsal segments (see figure A) of the fourth leg on the right side are bent downward sharply and are out of focus with the tibia. The tibia of the right metathoracic leg is above but so near the level of the tibia of the fourth leg that the two tibias appear to belong to the same leg. Unfortunately the specimen had been placed in potassium hydroxide so we do not know if the extra legs had muscles, though there are some strands of tissue remaining in them that are in the proper position for muscles. The specimen is in the Cornell collection.—JOHN SANJEAN AND B. V. TRAVIS, *Cornell University, Ithaca, New York. (Present address of Mr. Sanjean, Zoology Department, University of British Columbia, Vancouver, Canada.)*

ADDITIONAL INFORMATION PERTAINING TO THE INCIDENCE OF HEARTWORMS AND INTESTINAL HELMINTHS IN STRAY CATS AND DOGS IN BERGEN COUNTY, NORTHERN NEW JERSEY

Although much information, based upon autopsy findings, is available concerning the incidence of heartworms and intestinal helminths in stray dogs in various regions of the United States, there appears to be a scarcity of data as to the incidence of these particular parasites in stray cats found in the same localities.

Supplementary to a previously published report (Mann and Fratta, 1952, J. Parasit. **38:** 496–497), the writer, during 1954–55, had the opportunity to examine an additional 300 cats and 100 dogs in the Bergen County, northern New Jersey area. The animals were autopsied following euthanasia. The entire gastrointestinal tract, heart, and lungs of each animal were thoroughly searched for adult worms. Whenever possible, blood samples were checked by the concentration method of Stubbs and Live (1938, J. Amer. Vet. Med. Assn. **92:** 686) for microfilariae. Fecal examinations were made on a mixture of both cecal and intestinal contents using the saturated saline-centrifuge method.

The results, found in Table I., show that 72.0 percent of both the cats and the dogs examined

TABLE I.—*Parasites found in 300 cats and 100 dogs in Bergen County, northern New Jersey*

Parasite	No. cats positive	No. dogs positive
Ancylostoma caninum	57	22
Dipylidium caninum	97	32
Dirofilaria immitis	—	4
Taenia pisiformis	—	4
Taenia taeniaeformis	43	—
Toxascaris leonina	30	5
Toxocara canis	—	9
Toxocara cati	126	—
Trichuris vulpis	—	43
Total % positive animals	72	72

harbored parasites. It is interesting to note that heartworms, *Dirofilaria immitis*, were found in four of the dogs whereas none were observed in any of the cats. Although the dog and cat fleas, *Ctenocephalides felis* and *C. canis*, have been incriminated as potential vectors of *Dirofilaria* (Summers, 1940, Proc. Soc. Exper. Biol. and Med. **43:** 448–450) and recent work (Mann and Fratta, 1953, J. Parasit. **39:** 139–144) has shown that adult heartworms will live and propagate in the cat, no explanation can at present be given as to why this particular parasite was not found in any of the 300 cats.

Trichuris vulpis and *Dipylidium caninum* were the two most common parasites found in the dogs whereas the majority of the cats harbored Ascarids and/or *Dipylidium caninum*. The incidence of *Dipylidium caninum* (32.0 percent) in these stray animals emphasizes its importance from the standpoint of public health, inasmuch as this cosmopolitan tapeworm has been reported from man on over 250 occasions (Wright, 1947, Ann. N. York Acad. Sc. **48:** 553–576).

In conclusion, it should be noted that whipworms, *Trichuris* sp. were not found in any of the cats.—PHILLIP H. MANN, *Department of Health, Borough of Fort Lee, New Jersey.*

HELMINTH PARASITES OF SOME FUR-BEARERS OF OREGON

A limited survey of the incidence and abundance of helminth parasites in fur-bearers of Oregon was undertaken by the authors with the cooperation of the Oregon State Game Com-

mission and Dr. R. W. Macy of Reed College during the 1950–51 trapping season. A total of 64 animals were examined: 34 muskrats, 9 beaver, 11 mink, 2 weasels, 1 skunk, 6 otters, and 1 raccoon. All animals were trapped within 50 miles of Portland, Oregon. The helminths were identified by the authors with the exception of the ACANTHOCEPHALA which were identified by the late Dr. H. J. Van Cleave.

Muskrat, *Ondatra zibethica*. In general, the number of helminths present in muskrats was small, less than 50 per host, and of no apparent harm. The only exceptions were two muskrats from Clatskanie which contained, respectively, an estimated 100 and 300 immature cestodes, *Hymenolepis sp.*, in the small intestine. In addition, the following helminths were found, the number of hosts infected being given in parentheses: *Echinostoma revolutum* (12), *Euryhelmis pacificus* (1), *Q. quinqueserialis* (11), *Notocotylus sp.* (4), *Hymenolepis oregonensis* (20), *Hymenolepis sp.* (4), *Taenia taeniaeformis* (cysticercus) (9), *Trichuris opaca* (2). The *Notocotylus sp.* from the colon most closely resembled *N. attenuatus*, differing from it in having 8–10 coils of the uterus cephalic to the vitellaria.

Beaver, *Castor canadensis*. The 9 beavers examined were from the Clackamas River near Estacada. The trematode, *Stichorchis subtriquetrus*, was encountered in seven animals and was the most abundant helminth, more than 100 being present in some hosts. The maximum number of *Castorstrongylus castori* observed in one host was 17. Five animals were infected with *C. castori* and two with *Travassosius americanus*.

Mink, *Mustela vison*. *Alaria mustelae* was the principal helminth encountered, 10 animals being infected. An estimated 300 *A. mustelae* occurred in two of the mink from Columbia County. Two mink from Clackamas County had infections of *Euryhelmis pacificus*, *E. squamula*, *Nanophyetus salmincola*, and *Metagonimoides oregonensis*. The latter has apparently not been previously reported from mink. *Taenia tenuicollis* was found in two mink from Columbia County.

Weasel, *Mustela frenata*. Six *Alaria mustelae* were found in one weasel and six nematodes of the genus *Molenius* in the other.

Skunk, *Mephitis mephitis*. The only skunk examined came from Clackamas County. It contained six *Ascaris columnaris*, another nematode identified as a species of *Molenius* and several hundred cestodes of the genus *Oochoristica*.

Otter, *Lutra canadensis*. Six otters, two from the Columbia River in Multnomah County and four from the Clackamas River, were examined but no helminths were encountered.

Raccoon, *Procyon lotor*. The single raccoon taken in Multnomah County contained about 100 hookworms, *Arthrocephalus lotoris*, in the intestine and two immature ACANTHOCEPHALA, *Centrorhynchus* sp. in the colon.—CLYDE M. SENGER, *Montana Agricultural Experiment Station, Montana Veterinary Research Laboratory and Animal Disease and Parasite Research Branch, A. R. S., U. S. D. A., Bozeman, Montana,* AND KENNETH A. NEILAND, *Department of Parasitology, School of Public Health, Johns Hopkins University, Baltimore, Maryland.*

ISOSPORA BUTEONIS HENRY IN THE GOSHAWK (*ACCIPITER GENTILIS*)

On September 28, 1952, Dr. C. H. Bigland, Veterinary Pathologist of Edmonton, Alberta, submitted a portion of the intestine of a female goshawk (*Accipiter gentilis*) to Dr. A. A. Kingscote of the Ontario Veterinary College at Guelph, Ontario. Preliminary examination by Dr. Kingscote revealed that it was infected with a coccidium. The material was sent on to the Ontario Department of Lands and Forests' Wildlife Research Laboratory at Maple, Ontario, where the infective organism was identified as *Isospora buteonis* Henry. Apparently this is the first record of its occurrence in the goshawk.

The epithelial surface of the sample of intestine was scraped, especially in the region of the raised necrotic foci and the oocysts in the scrapings were concentrated by flotation in a saturated solution of sodium nitrate.

Comparison with the diagnostic characteristics of seven species of avian *Isospora* listed by Boughton (Boughton, Boughton and Volk, 1938, Ohio J. Sc. **38**: 149–163) revealed that only *I. buteonis* Henry resembled the unknown material sufficiently to be considered the same species.

Boughton *et al.* (1938) list *I. buteonis* from four hawks and two owls: Cooper's hawk (*Accipiter cooperi* (Bonaparte)), the red-tailed hawk (*Buteo borealis calurus* Cassin), Swainson's hawk (*Buteo swainsoni* Bonaparte), sparrow hawk (*Falco s. sparverius* Linn.), the short-eared owl (*Asio f. flammeus* (Pontoppidan)), horned owl (*Bubo virginianus pallescens* Stone).

The record for the sparrow hawk is probably an error. Henry (1932, Univ. Calif. Publ. Zool. **37**: 291–300) states that although she examined specimens of the sparrow hawk, she found

no evidence of infection with *Isospora buteonis*.—JOAN HOLLING, *124 Pim St., Sault Ste. Marie, Ontario, and* C. DAVID FOWLE, *Ontario Department of Lands and Forests, R. R. 2, Maple, Ontario.*

CHIGGERS ASSOCIATED WITH SQUAMOUS PAPILLOMATA ON A PEROGNATHUS MOUSE

On August 23, 1954 several specimens of *Perognathus pernix pernix* Allen were collected 25 kilometers southwest of Navojoa, Sonora, Mexico by Raymond E. Ryckman, Dean Spencer, and C. P. Christianson. When these animals were inspected for ectoparasites five wart-like structures were seen and removed from the rump region of one mouse. Microscopical inspection of the preserved growths indicated that they were heavily parasitized by mites. These mites were identified as *Trombicula potosina* and *T. panamensis* by Deane P. Furman and Douglas Gould. Dr. Furman (*in litt.*) states, "I have seen a similar reaction in wild birds such as the California road-runner and also quail, following chigger attachment; usually numerous chiggers are attached at one particular site and are associated with these growths." This is believed to be the first report of *T. potosina* from the state of Sonora, Mexico.

These growths were diagnosed as squamous papillomata of the skin. They consisted of a polypoid structure covered with benign stratified squamous epithelium which in several places showed papillary projections and elsewhere keratin-filled crypts. The stroma was dense connective tissue infiltrated with lymphocytes.—RAYMOND E. RYCKMAN, *Department of Entomology, School of Tropical and Preventive Medicine, and* JOHN C. ROOS, *Department of Pathology, School of Medicine, College of Medical Evangelists, Loma Linda, California.*

ARTHROPOD ECTOPARASITES OF RICHARDSON'S GROUND SQUIRREL

Arthropod ectoparasites of Richardson's ground squirrel, *Citellus richardsonii richardsonii* (Sabine), were collected in southern Saskatchewan as part of a survey to locate enzootic foci of sylvatic plague, tularaemia, and Rocky Mountain spotted fever during July and August of 1950 to 1952 inclusive. Southern Saskatchewan is herein described as the region south of the 52nd parallel. Ground squirrels were obtained from large areas surrounding 32 widespread localities within the designated portion of the province.

The ectoparasites were removed from 1,762 ground squirrels and consisted of 4,461 fleas, 271 lice, and 5,011 acarines. The latter comprised 2,161 ticks and 2,850 mites of which 437 were trombiculids. About 90 percent of the ectoparasites were submitted to the Laboratory of Hygiene, Kamloops, B. C., for use in isolating the aetiologic agents of plague, tularaemia, and spotted fever. Except for a few gamasid mites that could not be classified beyond subfamily, the remaining 10 percent of the ectoparasites were identified to genus or species and are reported here.

Siphonaptera	Other hosts in Saskatchewan
Catallagia decipiens Rothschild	*Microtus, Onychomys*
Foxella ignota albertensis (Jordan and Rothschild)	*Thomomys*
Hystrichopsylla dippiei Rothschild	*Peromyscus*
Monopsyllus wagneri systaltus (Jordan)	*Clethrionomys, Microtus, Mus, Onychomys, Peromyscus, Thomomys*
Neopsylla inopina Rothschild	*Citellus franklinii, Peromyscus, Mustela*
Opisocrostis labis (Jordan and Rothschild)	*C. franklinii, Cynomys, Mustela*
Opisocrostis tuberculatus tuberculatus (Baker)	*Citellus tridecemlineatus, Cynomys*
Oropsylla rupestris (Jordan)	*Citellus franklinii, Cynomys, Lepus, Mustela*
Rectofrontia fraterna (Baker)	*Lepus, Mustela, Onychomys*
Thrassis bacchi (Rothschild)	*Citellus tridecemlineatus, Cynomys, Mustela*

Anoplura

Neohaematopinus laeviusculus (Grube) (nymphs and adults)

Acarina

Dermacentor andersoni Stiles (larvae and nymphs)
Ixodes sculptus Neumann (larvae, nymphs, and adults)
Euhaemogamasus barberi (Ewing)
Haemolaelaps glasgowi (Ewing)
Ischyropoda armatus Keegan
Euschöngastia sp.
Trombicula sp.

In conclusion, it is of interest to note that no disease producing organisms were isolated from any of the ectoparasites of the ground squirrel. However, in another phase of the work a virulent strain of *Pasteurella tularensis* was discovered in "drag" ticks (adult *Dermacentor andersoni*) collected around Maple Creek and Govenlock in southwestern Saskatchewan in June of each year during the survey.

The information reported here summarizes a thesis presented to McGill University in partial fulfillment of the requirements for the degree of Master of Science, Parasitology. The work was supported by the Saskatchewan Department of Public Health through a Dominion-Provincial Health Grant.—G. D. BURGESS, *Entomology Laboratory, Canada, Department of Agriculture, Saskatoon, Saskatchewan.*

THE INCIDENCE OF OPALINID INFUSORIANS IN TWO SPECIES OF TADPOLES

During the period from May to August, 1955 two species of anuran tadpoles were collected in the vicinity of Ithaca, New York. Smears of the gut were made within a few hours after collection, fixed in Schaudinn fluid, stained with Heidenhain's iron hematoxylin, and mounted in balsam.

Identification to species was based chiefly on body shape and size, and the number and size of the nuclei (Metcalf, 1923, Bull. 120, U. S. Nat. Mus., 1–484). Seventy-six percent of the *Bufo americanus* and 28 percent of the *Rana clamitans* tadpoles were infected with opalinids. The species in *Bufo*, which was considered to be *Opalina obtrigonoidea*, averaged 60 microns in length, 46 microns in width, and had nuclei averaging 4.2 microns in diameter. In *Rana* the species which was considered to be *O. ranarum* averaged 120 microns in length, 80 microns in width, and the nuclei averaged 5.4 microns in diameter.

Both the frog and toad tadpoles harbored opalinids which were smaller than the typical forms mentioned and measured from 54 to 112 microns in length and 18 to 50 microns in width. A tapering, pointed posterior end was characteristic and nuclei numbered 8 to 14 in an individual. Similar forms were described as *O. larvarum* by Metcalf (1940, Proc. U. S. Nat. Mus. **87**: 465–634) who suggested that they probably represent a transitional stage in the developmental cycle of *O. ranarum*. From the present study it appears that *O. larvarum* might represent an early stage of either of the opalinid species observed in the frog and the toad tadpoles.—CARLITA L. NESSLINGER, *Cornell University, Ithaca, New York.*

SOME HELMINTH PARASITES OF RODENTS FROM LOCALITIES IN MARYLAND AND KENTUCKY

During 1954, 369 rodents were examined at this laboratory for parasites of the liver, lungs, and alimentary tract. These animals were collected during an ectoparasite training survey by members of the 498th Medical Company and the 562nd Medical Detachment. One group of rodents was taken from the vicinity of Fort George G. Meade, Maryland from January through June and from September through December. Another group was collected at Fort Knox, Kentucky from June through early September. No attempt was made to determine the seasonal distribution of the parasites, or to correlate their occurrence with the sex or age of the host.

From the Fort Meade area 213 rodents were taken; 104 of these harbored at least one species of helminth. Of the 156 rodents collected at Fort Knox, 75 harbored at least one helminth species.

A listing of the animals examined and their respective parasites follows. Numbers in parentheses following the names of the parasites indicate the number of hosts harboring the helminth species.

FORT MEADE MATERIAL—213 rodents examined; 104 infected.

Microtus pennsylvanicus—26 of 35 infected

CESTODES: *Andrya macrocephala* (3), *Paranoplocephala infrequens* (3), *?Hymenolepis sp.* (2), unidentified immature cestodes (2).

NEMATODES: *Trichuris* sp. (3), *?Trichuris* sp. (1), unidentified immature trichurid (1), *Syphacia obvelata* (8), *Nematospiroides* sp. (14), *?Nematospiroides* sp. (2), unidentified trichostrongylids (2), unidentified nematodes (1).

Mus musculus—20 of 78 infected.

CESTODES: *Cysticercus fasciolaris* (1).

NEMATODES: *Syphacia obvelata* (5), *Nematospiroides dubius* (3), *?Longistriata* sp. (1), unidentified trichostrongylids (1), *Mastophorus muris* (3), unidentified larval nematodes (7).

Peromyscus leucopus—44 of 82 infected.

TREMATODES: *Brachylaemus peromysci* (1).

CESTODES: *Cysticercus talpae* (4), Cysticercus of *Paruterina* sp. (2).

NEMATODES: *Capillaria* sp. (2), unidentified trichurids (2), *Syphacia peromysci* (17), *Nematospiroides dubius* (3), *Rictularia coloradensis* (17), immature *Physaloptera* sp. (1), unidentified adult nematodes (1), unidentified larval nematodes—2 types (9).

Pitmys pinetorum—1 of 1 infected.

NEMATODES: *Trichuris opaca* (1), *Syphacia* sp. (1).

Rattus norvegicus—13 of 14 infected.

CESTODES: *Hymenolepis nana* var. *fraterna* (4), *Cysticercus fasciolaris* (5).

NEMATODES: *Strongyloides* sp. (1), *Heterakis spumosa* (6), *Nippostrongylus muris* (1), unidentified nematode (1), unidentified larval nematode (1).

Zapus hudsonius—0 of 3 infected.

FORT KNOX MATERIAL—156 rodents examined; 75 infected.

Microtus pennsylvanicus—37 of 67 infected.

CESTODES: *Andrya macrocephala* (6), *Andrya* sp. (1), *Paranoplocephala infrequens* (1), *?Hymenolepis* sp. (1), unidentified immature cestode (1).

NEMATODES: *Syphacia obvelata* (32), unidentified trichostrongylids (2).

Mus musculus—8 of 30 infected.

TREMATODES: *Brachylaemus peromysci* (1) ?.

NEMATODES: *Aspicularis tetraptera* (1), *Syphacia obvelata* (4), *Rictularia coloradensis* (1) ?, unidentified nematode larvae (2).

Peromyscus leucopus—27 of 53 infected.

TREMATODES: *Brachylaemus peromysci* (2).

CESTODES: Cysticercus of *Paruterina* sp. (1).

NEMATODES: *Aspicularis tetraptera* (1), *Syphacia peromysci* (12), *Rictularia coloradensis* (16), unidentified nematode larvae (2).

Rattus norvegicus—3 of 6 infected.

CESTODES: *Cysticercus fasciolaris* (1), *Hymenolepis diminuta* (2).

NEMATODES: *Heterakis spumosa* (2), unidentified nematode larvae (1).

Acknowledgement is given to members of the 498th Medical Company and the 562nd Medical Detachment for collection and autopsy of the rodents; to Dr. T. T. Harris, former Commanding Officer of the 498th Medical Company, for photographical assistance; and to Messrs. A. McIntosh, J. T. Lucker, and A. Goldberg of the Animal Disease and Parasite Research Branch, USDA, Beltsville, Maryland for identification of some of the helminths, aid in identification of others, and for general technical advice.—J. E. HALL AND B. SONNENBERG, *Second Army Area Medical Laboratory,* AND J. R. HODES, *498th Medical Company, Fort George G. Meade, Maryland.*

AN EFFICIENT DEVICE FOR EXPOSING MICE TO SCHISTOSOME CERCARIAE AND HOLDING SMALL ANIMALS FOR POST MORTEM EXAMINATION

The device described below has proved more satisfactory for exposing mice and autopsying small animals than any other we have encountered. The chamber described by Stirewalt and Bronson (1955, J. Parasit. **41**: 328) has been tried. In comparing the two types the one described here was preferred because: (1) it is more easily made by an unskilled worker from materials readily available; (2) the tail does not need to be taped in place and the mouse is more easily removed; (3) rotation of the mouse in the tube does no harm.

The baseboard is made from a rectangular sheet of ¼ inch transparent plastic. The size of the plastic sheet can be varied to suit individual needs. The one used in this laboratory measures 20 by 34 inches. Round holes 1⅛ inches in diameter with their centers 3¼ inches apart are made in the plastic sheet (Fig. 1, A). Another hole ⅛ inch in diameter (Fig. 1, B) is centered in any one direction ⅜ inch from the edge of each 1⅛ inch hole. A plastic spring type clothespin is centered beneath each 1⅛ inch hole and attached with a flat-headed stove bolt with head countersunk in the plastic (Fig. 2). When being used for infecting animals, the clothespin holds the tube containing the cercarial suspension.

The mouse chamber and its use for infecting mice with schistosomes by the tail immersion method are illustrated in Figure 3. The baseboard is supported above the table top by means of any suitable support, such as detachable legs. Each mouse chamber is made from two plastic 50 ml. centrifuge tubes. One, with its lip and most of its curved end removed, is split longitudinally and its edges overlapped slightly so that it can be inserted inside the other for reinforcement. Most of the curved end of the outer tube is also removed and its mouth is inserted in a suitably bored square of 7/16 inch plastic (Fig. 3). Small holes for ventilation are made in the walls of the double tube. The curved end of the double tube is closed with a circle

of 7/16 inch plastic bored in the center with a ¼ inch diameter hole. This plastic circle is the one removed from the square in which the mouth of the tube is inserted. A piston of ¼ inch plastic (the circle cut from the baseboard) with a 7 cm. length of 5 mm. glass tubing cemented into a hole in its center is inserted as shown, the glass tubing passing through the ¼ inch hole in the tube closure. A screw eye is added so that the piston can be locked at any suitable position, depending on the size of the mouse. The plastic square containing the mouth of the tube is hinged to a similar sized square of ¼ inch plastic containing a slot through which the tail passes by means of a hinge made from a spring-type woman's wave clip. The wave clip spring serves to hold the chamber closed, and in practice a rubber band is used on the side opposite the hinge for additional security. An anchor is fashioned from the lower half of the

FIG. 2: TYPICAL SET OF HOLES WITH CLOTHESPIN

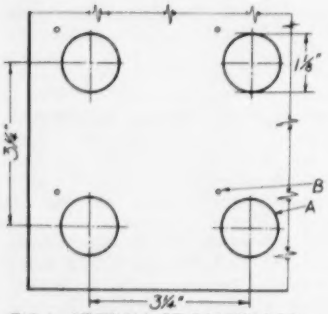


FIG. 1 SECTION OF BASEBOARD

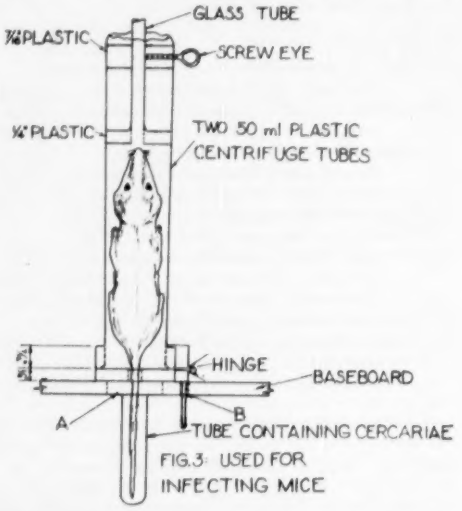


FIG. 3: USED FOR INFECTING MICE

wave clip hinge. To do this, a strip is cut along one of the lower edges of the clip. The cut extends to the edge of the moveable piece of plastic. The strip is bent at right angles to the surface of the plastic square and pressed into a rodlike shape. The insertion of this rod into hole B in the baseboard serves to anchor the chamber. A small metal rod soldered to the wave clip can be substituted for the anchor described above.

When the baseboard is not being used for the exposure of animals, it can be turned over and used to hold small animals for autopsy. The animals are held firmly in place with a clothespin on each leg.—Luis A. Berrios-Duran, *Tropical Research Medical Laboratory, U. S. Army, San Juan, Puerto Rico.*

APPARENT EXTRINSIC VARIATION IN *KNEMIDOKOPTES PILAE* LAVOIPIERRE AND GRIFFITHS, 1951 (ACARINA: SARCOPTIFORMES)

Mites of the family Sarcoptidae are usually divided into two groups in keys to the genera: those with a dorsal anus and those with a terminal anus. Recent evidence has been encountered which indicates that this character cannot be relied upon in all cases.

The author examined some specimens of *Knemidokoptes* sp. taken in September, 1953, by E. J. Hansens in Liberty Corners, New Jersey, from a budgerigar, *Melopsittacus undulatus* (Shaw, 1805), suffering from scaly leg. These mites fitted the description of *K. pilae* Lavoipierre and Griffiths (1951, Ann. Trop. Med. Parasitol. **45:** 253-254) which species was described from a case of budgerigar scaly leg. Members of the genus *Knemidokoptes* are mange mites of such avian hosts as poultry, starlings, pigeons and canaries, where they cause scaly leg, depluming itch and other skin disorders. They may be separated from other sarcoptid mites by the presence of sclerotized apodemes beneath the scutum, absence of peg-like spines on the idiosoma, and tarsi of females without ambulacral suckers. *K. pilae* may easily be distinguished from all other species of the genus: by the origination of the paired adscutal setae at a common base; by the scutum being completely punctate and having a definite border; by the distinct angulation of the apodemes of coxae I; by the equal length of claw-like projections of tarsi III and IV; and by other characters such as body size and integumental pattern.

One unusual feature was noted: the anal position was dorsal in all adult females. To the author's knowledge, no description of a species of *Knemidokoptes* has ever included this singular character (hence, present reliance on terminal anus as a generic character in *Knemidokoptes*). In the original description of *K. pilae* no information was given regarding the position of the anus. Through the courtesy of Dr. G. Owen Evans, of the British Museum (Natural History), several specimens of *K. pilae* were obtained and compared with the New Jersey specimens. Both groups showed dorsal ani and were obviously identical in other respects.

In June, 1955 a budgerigar which had a wart-like growth on its cere was received from a breeder in Charlotte Hall, Maryland. The growth, which extended from the cere to the feathered area between the cere and the eye, contained mites which closely resembled *K. pilae*. Except for slightly larger mean body size of this mite over that of typical *K. pilae*, comparisons of series of each revealed only one morphological difference: the anus was clearly terminal in the adult female specimens from the cere. In both forms, the larvae possessed terminal ani and were indistinguishable.

It is possible that the specimens from the leg and those from the cere represent different species or subspecies which originally parasitized different species of birds, only recently utilizing *M. undulatus* as a common host. Budgerigars are bred and sold as pets, and often are transported, pooled and housed with other bird pets such as canaries and parrots. Lavoipierre and Griffiths refer to a mite infesting canaries in South Africa which causes scaly leg (Kaschula, 1950, J. S. Afr. Vet. Med. Ass. **21**: 117), and from comparisons of photomicrographs they indicate that it is possibly the same as *K. pilae*.

On the contrary, the larvae of both forms show no differences, the adults obviously differ only in size and in anal position, and they both occur on the same species of host within the same geographical range. It is, therefore, also possible that the difference is of a varietal nature. The cere and the leg of the host, though both covered with epidermis, offer a dissimilar environment to the invading mite, the former being soft and spongy, the latter cornified and scaly. If the differences noted are a response to the heterogeneity of the environment, then they are an example of ecophenotypic variation. It is not now possible to say whether the forms are genetically distinct or ecophenotypes. At attempt to transmit the mite by introducing the infected bird to a cage of normal ones has so far proven unsuccessful.

Regardless of the taxonomic status of the two forms, anal position as a generic character is not infallible. This would indicate that a reevaluation of generic concepts in the SARCOPTIDAE should be undertaken. It should also be noted that this represents a new locality record for *K. pilae*, originally found in Great Britain.—CONRAD E. YUNKER, *Department of Zoology, University of Maryland, College Park, Maryland.*

KLOSSIELLA EQUI:—CORRECTION

In a recent publication (1955, J. Parasit. **41**: 285–288) *Klossiella equi* was described by the writers as a new species of KLOSSIELLIDAE found in the kidney of the American jack. Shortly after the appearance of this article, it was found that the name *Klossiella equi* Seibold and Thorson, 1955 was both a homonym and a synonym of *K. equi* Baumann, 1946, since Baumann (1946, Wien, Tierartzl. Monatschr. **33**: 257–260) had used the name to describe a *Klossiella* in the kidney of a horse. Dr. Baumann was kind enough to send us some of his tissue sections and a comparison was made with our material. In our opinion, the organisms in Baumann's case and ours are identical. Therefore, the name *Klossiella equi* Baumann, 1946 is the proper one for the species found in the American jack.—H. R. SEIBOLD AND RALPH E. THORSON, *Department of Pathology, School of Veterinary Medicine, Alabama Polytechnic Institute, Auburn, Alabama.*

ANNOUNCEMENT

By mail vote Council has elected Dr. Leon Jacobs, Laboratory of Tropical Diseases, National Institutes of Health, Bethesda 14, Maryland, Chairman of the Editorial Committee. Manuscripts submitted for publication in the Journal of Parasitology should be sent to Dr. Jacobs at the above address.

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